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EVALUATION OF THE IAA-PRODUCING ABILITY OF SOME ENDOPHYTIC BACTERIA ISOLATED FROM *Panax pseudoginseng* ROOTS

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ABSTRACT

The objective of this research was to evaluate the Indole-3-acetic acid (IAA) producing ability of isolated endophytic bacteria from *Panax pseudoginseng* roots through *in vitro* culture conditions. As a result attained, 16 endophytic bacterial strains, including both gram-positive and gram-negative bacteria were isolated from the collected roots of *P. pseudoginseng* and all of these isolates could produce IAA with different concentrations, which ranged from 4.61 µg/mL to 85.11 µg/mL, respectively. Among the strains, NN1 and NN6 resulted in the most productive IAA by 85.11 µg/mL and 69.29 µg/mL, respectively. Underlay of NA liquid medium combined with L-tryptophan, 6-day dark incubation with pH 6-8 showed a better synthesis of IAA. While, KNO_3 and $(NH_4)_2 HPO_4$ were considered the optimal nitrogen sources for IAA production of NN1 (56.36 µg/mL and 55.52 µg/mL) and NN6 (54.17 µg/mL and 42.93 µg/mL, respectively) strains, dextrin and saccharose were the best suitable carbon sources for bacterial isolates NN1 (59.17 µg/mL) and NN6 (67.24 µg/mL). The attained results suggest that the diversity of endophytic bacterial species in *P. pseudoginseng* roots and their IAA-producing potential are largely based on cultural techniques. Besides, the findings of this study can serve as a foundation for developing natural bioactivity products to improve the productivity of ginsengs.

Keywords: *Endophytic bacteria, Indole-3-acetic acid (IAA), carbon and nitrogen sources, Panax pseudoginseng, plant growth promotion.*

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1. INTRODUCTION

Endophytic bacteria reside within living plant tissues without any harmfulness or nutrient competition with host plants [1]. These endophytic microorganisms can even restrain disease expression caused by some plant pathogens and promote plant growth [1, 2]. According to previous studies, the community of endophytic bacteria mostly exists in the rhizosphere or root surface, but they can break the endodermal barrier and move from the root cortex into the vascular system, and then inhabit tubers, stems, leaves, and other parts of the plants [3, 4]. Endophytic bacterial species are involved in promoting plant growth by diverse mechanisms, such as solubilizing mineral phosphates, fixing nitrogen, supplying micronutrients, producing siderophore, activating photosynthetic process, stimulating endogenous hormone activity, and promoting beneficial plant-microbe symbiosis [3, 5, 6, 7].

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Furthermore, bacterial endophytes also play an important role in producing antibiotics and secondary metabolites to enhance the plant defence systems against soil pathogens and various environmental stresses [7, 8]. Several phytohormones including IAA, gibberellins, and cytokinins synthesized by the stem and root endophyte bacteria strains enable to promoting plant physiological processes and protect the host plant from biotic and abiotic stresses [9, 10]. IAA is an important endogenous auxin hormone for cell division and enlargement contributing to the formation and elongation of plant roots, root hairs, and branch roots [9-11].

P. pseudoginseng has been known as one of the most precious oriental herbs. In Vietnam, *P. pseudoginseng* is called "Tam That" which implies "three-seven roots" and it is usually used as a health tonic in traditional medicine. According to popular remedies, *P. pseudoginseng* can help to treat some human diseases, such as insomnia, nervous depression, tumors, cancer, cardiovascular neurasthenia, lumbar pain, haemorrhage, and nosebleeds [12, 13]. The studies have reported that this plant also possesses a high content of ginseng saponins, the most important phytoconstituent in *Panax* species, such as ginsenosides-Rb_3, -Rd, -Re, -Rg_1, -Rt_1, -F_{11} and -F_8 [14, 15], respectively. In recent years, due to the high increase of using the demand for medicinal plants, *P. pseudoginseng* is facing over-exploitation. Besides, present sources of *P. pseudoginseng* mostly depend on field cultivation, which requires time and farming techniques. To reduce the time of cultivation as well as shorten the growth cycle for harvesting, farmers usually use chemical reagents and fertilizers to enhance plant growth parameters. However, this farming method causes harmful impacts on the soil environment, it also affects the plant-beneficial microbes interaction, decreasing the accumulation of ginsenosides and the quality of *P. pseudoginseng* as well. To date, although numerous endophytic microbes from various crop plants have been isolated and assessed for their biocontrol and plant growth stimulation abilities, however, little available information on the characterization of bacterial endophytes from ginseng is reported. Therefore, this study aimed to examine the diversity of endophytic bacteria strains in *P. pseudoginseng* roots and evaluate their levels of IAA biosynthesis under the *in vitro* culture condition. The utility of some of these isolated bacterial endophytes as biocontrol reagents promoting plant growth will be estimated and further applied to develop natural bioactive products for improving the quality and productivity of *P. pseudoginseng*.

2. MATERIALS AND METHODS

2.1. Materials

The sample of *P. pseudoginseng* was collected in Lai Chau province and kept at Agricultural Genetics Institute (AGI), km 2, Pham Van Dong road, Co Nhue, Bac Tu Liem, Ha Noi, Vietnam.

The bacterial cultural mediums used in the study include: NA medium(g/L): pepton-5; NaCl-5; meat extract-2; yeast extract-3; agar-18; 1000 mL of H_2O. NBRIP culture (g/L): glucose-10; $Ca_3(PO_4)_2$-10; $MgCl_2.6H_2O$-5; $MgSO_4.7H_2O$, 0.25; KCl-0.2; $(NH_4)_2SO_4$, 0.1; 1000 mL of H_2O; pH 7.0. Basal mineral medium (g/L): K_2HPO_4-1.73; KH_2PO_4-0.68; $MgSO_4.7H_2O$-0.25; NaCl-4; $FeSO_4.7H_2O$-0.03; NH_4NO_3-1, $CaCl_2.2H_2O$ -0.02; glucose-5.

2.2. Methods

The root samples of *P. pseudoginseng* were collected from healthy *P. pseudoginseng* plants and temporarily stored at 4°C until conducting isolation. Endophytic bacteria isolation was performed following a method described by Chen *et al.* (2014) [16]. Briefly, to remove the soil, *P. pseudoginseng*'s roots were washed under water tap, then they were cut into small pieces and

soaked in 75% ethanol for 2.5 minutes. Root sterilization was continuously treated with 3% sodium hypochlorite for 2 minutes and 75% ethanol for 30 seconds. After each time of sterilizing treatment, root samples were washed again with distilled water. To make sure the root surface is entirely sterilized, washing samples of distilled water would be tested on the NA-contained Petri dishes without any living microorganisms after 2 days of 30°C incubation. Next, sterilized root samples were inoculated into NA medium using Petri dishes and these dishes were put in an incubator at 30°C. The development of endophytic microorganisms was observed after several days of culture. As soon as appearing the development of individual single colonies, they were picked up and transferred to a new cultural medium and kept at 4°C for further experiments.

Indole acetic acid (IAA) production: Endophytic bacterial samples were inoculated into 20 mL of NA liquid medium containing L-tryptophan. Afterward, the solution was shaken at 200 rpm, incubated at 30°C for 48 hours, and then centrifuged at 3000 rpm for 15 minutes. Finally, to determine the IAA concentrations of isolated bacterial strains, 1 mL of supernatant from centrifugation was mixed with 2 mL of Salkowski reagent and incubated for 30 minutes. The appearance of red color in test mixtures showed a synthesis of IAA from bacterial isolates and the concentration was determined according to the Vietnamese National Standard TCVN 10784: 2015 [17].

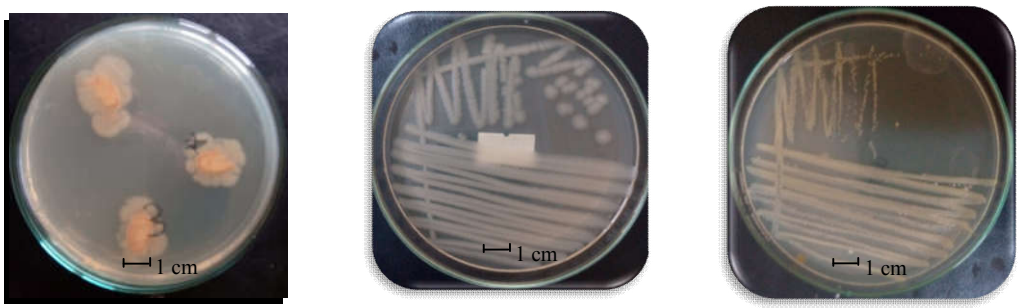
Effect of incubation time and pH on the production of IAA: In dark conditions, isolated bacterial strains were cultured in NA liquid medium supplemented with L-tryptophan (100 mg/L). The IAA content of each bacterial strain was separately measured every 24 hours of culture and in different pH values (ranging from 3.0 to 10.0). The measurement of IAA concentration was conducted following the Salkowski colorimetric method with the wavelength of ultraviolet absorption of the detector at 530 nm [17].

Effect of carbon and nitrogen sources on the production of IAA: The carbon sources were supplemented in basal medium (Trp^+NA liquid medium) including starch, lactose, dextrin, sucrose, D-sorbitol, and mannitol. The added sources of nitrogen were $(NH_4)_2SO_4$, NH_4Cl, NH_4NO_3, NH_4Cl, $(NH_4)_2HPO_4$, KNO_3, and peptone. Whereas, the controls only used basic mediums. The concentration of PO_4^{3-} is determined according to the method described above.

Statistical analyses: All the experiments were replicated three times. The data were analyzed by using Microsoft Excel ver 2016 and presented as the mean values.

3. RESULTS AND DISCUSSION

3.1. Isolating endophytic bacteria from the root of *P. pseudoginseng*



Isolation results NN1 Sample NN6 Sample

Figure 1. Endophytic bacteria isolated from the root of *P. pseudoginseng*

The sterilized samples of *P. pseudoginseng* roots were cultured in NA medium using Petri dishes and incubated at 30°C (Figure 1). In this experiment, a total of sixteen different endophytic bacteria were isolated.

Endophytic bacteria appeared from two root segments placed on the agar plate. Microbial samples were transplanted into LB medium for purification. Colonies of isolated bacteria samples expressed the diversity of shape, color, and size. Most of the colonies had milky white and yellow colors, with smooth surfaces. Among sixteen endophytic bacteria, there were 13 gram-positive bacteria and 3 gram-negative bacteria. Thus, these new strains of isolated endophytic bacteria may belong to different species or genera.

3.2. Evaluation of IAA synthesizing capacity of isolated bacterial strains under laboratory conditions

The results of IAA biosynthesis showed that all sixteen bacteria isolates were able to produce IAA (Figure 2). The highest contents of IAA were 85.11 µg/mL and 69.29 µg/mL produced by strains NN1 and NN6, respectively. Other bacteria samples synthesized lower IAA contents with different concentrations. According to the report of Tuyet and Giang [18], isolated twenty-one endophytic bacteria species from the turmeric's roots and almost all strains could synthesize IAA with various concentrations, ranging from 3.33 µg/mL to 65.38 µg/mL. Similarly, in the experiment of Hassan *et al.* (2017) [5], IAA contents synthesized by fungal and bacterial endophytes in the medicinal plant of *Teucrium polium* L. were 19.9 - 63.5 µg/mL and 4.1 - 23.4 µg/mL, respectively. From the roots of *Aloe vera*, Nguyen Van Giang *et al.* (2016) [6] found out 14 isolates of endophytic bacteria could synthesize IAA with contents from 16.49 - 36.32 µg/mL. In addition, Chandra *et al.* (2018) [19] also isolated two endophytic bacteria samples from *Stevia rebaudiana* rhizosphere CA1001 and CA2004 could produce very high contents of IAA, 91.7 µg/mL and 81.7 µg/mL, respectively. Our findings revealed that IAA biosynthesis from endophytic bacteria in *P. pseudoginseng*'s roots was quite popular and necessary for plant growth promotion. Among them, two isolates NN1 and NN6 showed the highest contents of IAA production, and they were selected for further analysis.

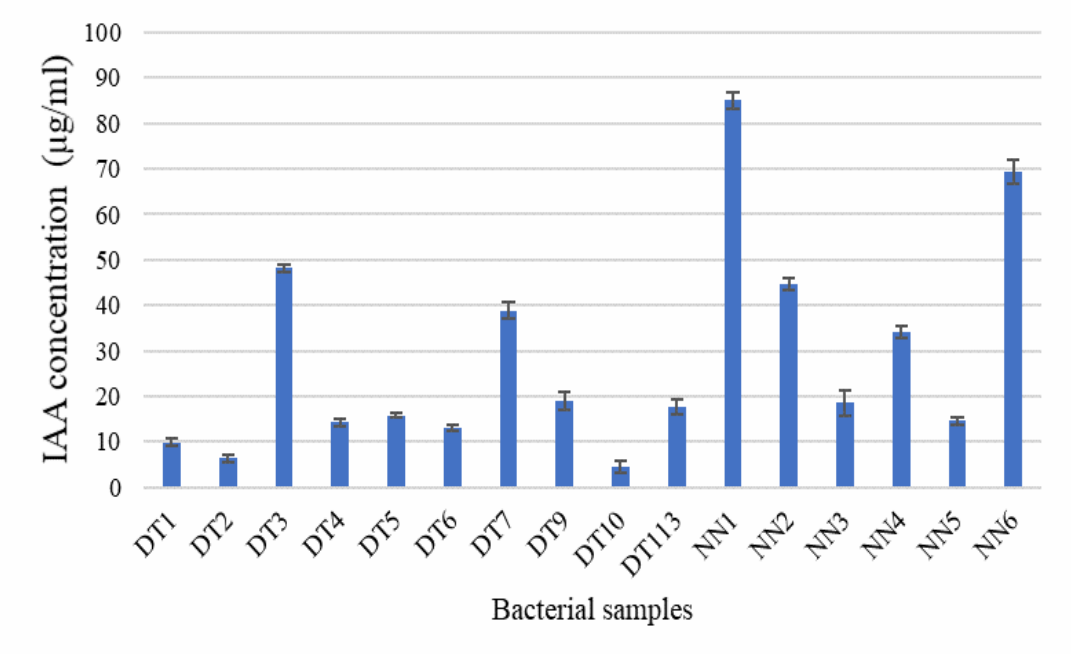


Figure 2. The amount of IAA production synthesized by endophytic bacteria

3.3. Effect of *in vitro* culture conditions on the ability of IAA production of NN1 and NN6 strains

3.3.1. Effect of incubation time

Generally, the content of IAA generated by NN1 and NN6 strains trends gradually increased in the first 6 days of culture (Figure 3). On the 6th day (after 144 hours), the concentration of IAA caused by NN1 and NN6 were the highest, comparing other days, 85.11 µg/mL and 69.29 µg/mL, respectively. However, on the 7th day, IAA content was reduced in both NN1 and NN6 strains (82.08 µg/mL and 64.22 µg/mL, respectively), suggesting that the capacity of IAA synthesis of NN1 and NN6 gets maximum value when nutrient sources are adequately supplied in a certain time of culture. On the contrary, in case of nutrient sources are insufficient or exhausted, the ability of IAA production of endophytic bacteria could be remarkably decreased. On the other hand, IAA-producing property also depends on the different characteristics of each bacterial species. Maximization of IAA content was determined the incubative time of *P. pseudoginseng* rhizobacteria in this study which is similar to several previous reports, but with diverse endophytic isolates from various plants. For instance, Kumari *et al.* (2018) [20] optimized IAA production (137.81 µg/mL) in rhizospheric bacteria of roadside weed, *Eragrostis cynosuroides* after 4-day incubation (96 hours) in JNFb$^-$ Trp$^+$ broth medium. Apine *et al.* (2011) [21] also reported that IAA synthesis caused by a bacterium *Pantoea agglomerans* strains from *Nicotiana tobacum* (leaf) explants were optimized under conditions as a medium containing meat extract and tryptophan, pH 7, and 2-day incubation in 30^{o}C.

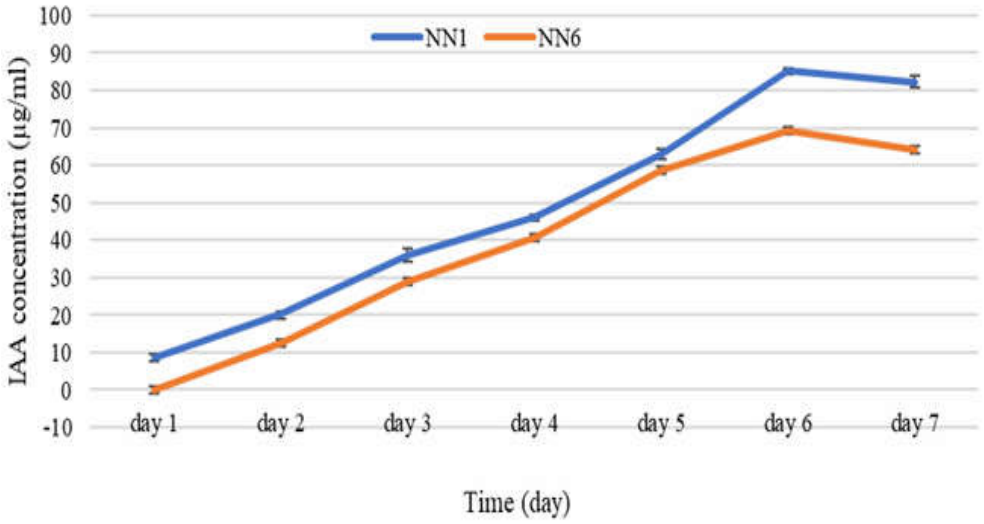


Figure 3. Effect of culture time on IAA synthesis ability

3.3.2. Effect of pH on IAA production of NN1 and NN6

The result exhibited all endophytic bacterial strains could produce IAA in the range of pH from 3.0 to 10.0. However, rhizobacterial strains cultured in Trp$^+$ NA liquid medium with the adjustment of pH 6.0-8.0 were assessed as the most appropriate for optimal producing IAA (Figure 4). Nita *et al.* (2011) [22] proved that the highest IAA content caused by *A. dazotrophicus* L1 strain was cultured in media with pH 6.0. In the experiment of Chandra *et al.* (2018) [19], bacterial isolates from *Stevia rebaudiana* rhizosphere including CA1001, CA2003, and CA2004 expressed the most synthesis ability of IAA contents ranging pH adjustment from 5.0 to 9.0, respectively. In other investigations, pH 6.0-7.0 was estimated as the most suitable and common adjustment for

rhizobacteria isolated from turmeric roots [18] and *Aloe vera* roots [6] to produce optimal IAA hormone.

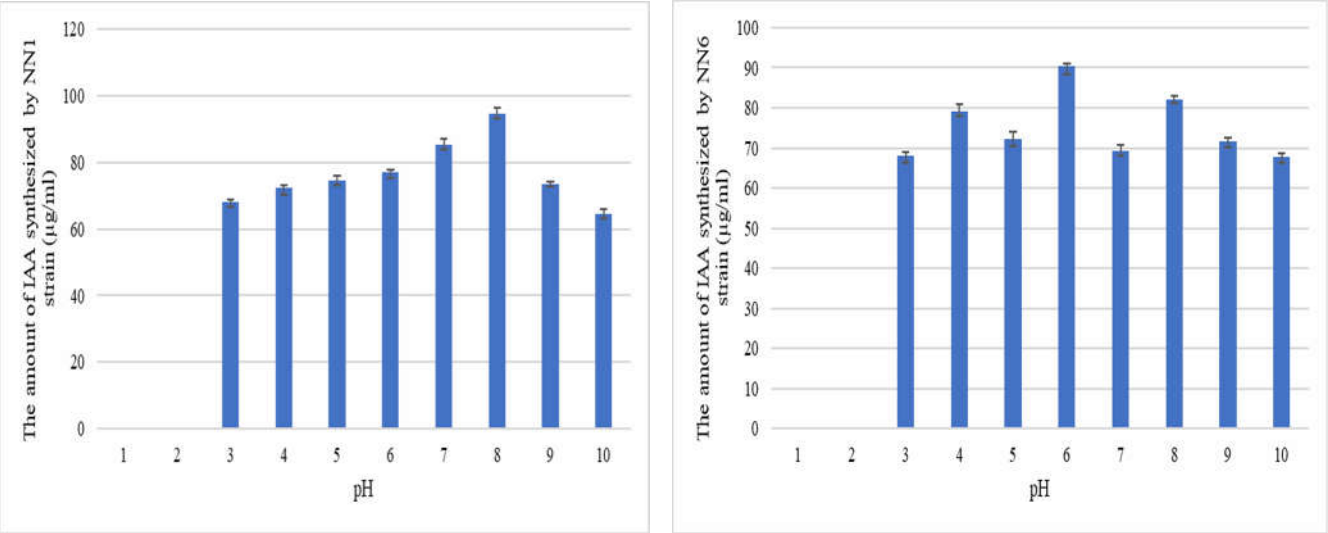


Figure 4. Effect of pH on the ability of IAA synthesis of NN1 (left) and NN6 (right)

3.3.3. Effect of carbon source on IAA production of NN1 and NN6

Carbon is one of the most important nutrient sources for endophytic microorganisms to synthesize the necessary components, essential enzymes or plant growth hormones. In this research, NN1 and NN6 strains cultured in Trp^+ NA medium were separately supplied with different carbon sources, including dextrin, lactose, sorbitol, saccharose, fructose, and xylose. Glucose was subjected to a carbon source used in the control sample.

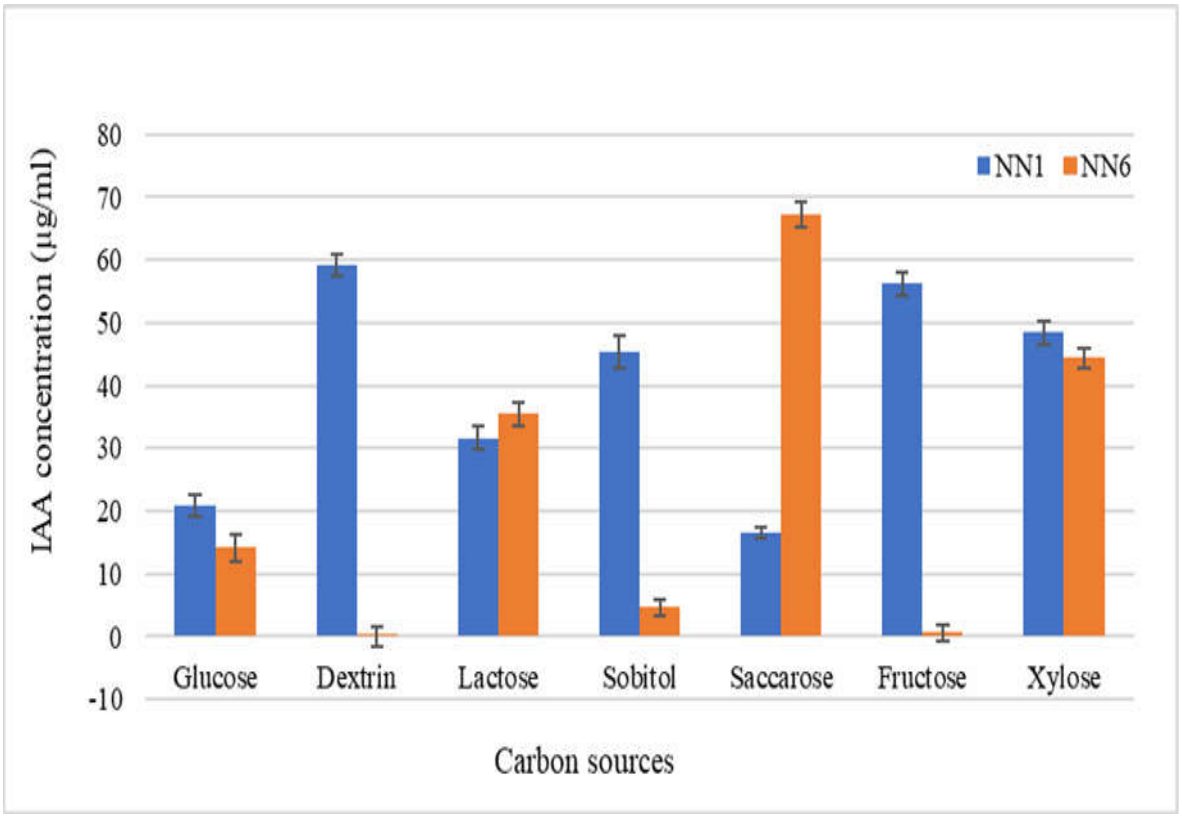


Figure 5. Effect of carbon source on IAA production of NN1 and NN6

The capacity of carbon absorption to produce IAA hormone in NN1 and NN6 strains are shown in figure 5. As a result, dextrin was revealed to be a suitable carbon source for NN1 when generating a maximum content of IAA (59.27 µg/mL). However, this carbon source virtually did not affect the IAA synthesis of NN6 (0.05 µg/mL). Nevertheless, saccharose was the appropriate carbon for NN6 strain to create the highest amount of IAA (67.24 µg/mL) and saccharose induced the inefficiency of IAA production in NN1 strain (16.63 µg/mL). Our results were in agreement with the report of Nita *et al.* (2011) [22] found that saccharose is a potential carbon source for the productive synthesis of IAA in *A. diazotrophicus* L1 strains from sugarcane.

3.3.4. Effect of nitrogen sources on the IAA production of NN1 and NN6

The influence of nitrogen sources including $(NH_4)SO_4$, NH_4Cl, NH_4NO_3, NH_4Cl, $(NH_4)HPO_4$, KNO_3, and peptone on the IAA-producing levels of NN1 and NN6 bacteria are shown in figure 6.

Generally, IAA content was synthesized by NN1 and NN6 strains which had significant differences between nitrogen sources. Supplement of the nitrogen sources KNO_3 and $(NH_4)_2HPO_4$ resulted in the highest contents of IAA production in both NN1 (56.36 µg/mL and 55.52 µg/mL, respectively) and NN6 (54.17 µg/mL and 42.93 µg/mL, respectively). This result is inconsistent with the results of Kumari *et al.* (2018) [20] who reported that in an environment containing KNO_3, the concentration of IAA was not high. However, Mohite *et al.* (2013) [23] concluded that a suitable source of nitrogen for synthesizing IAA of samples is KNO_3 and peptone.

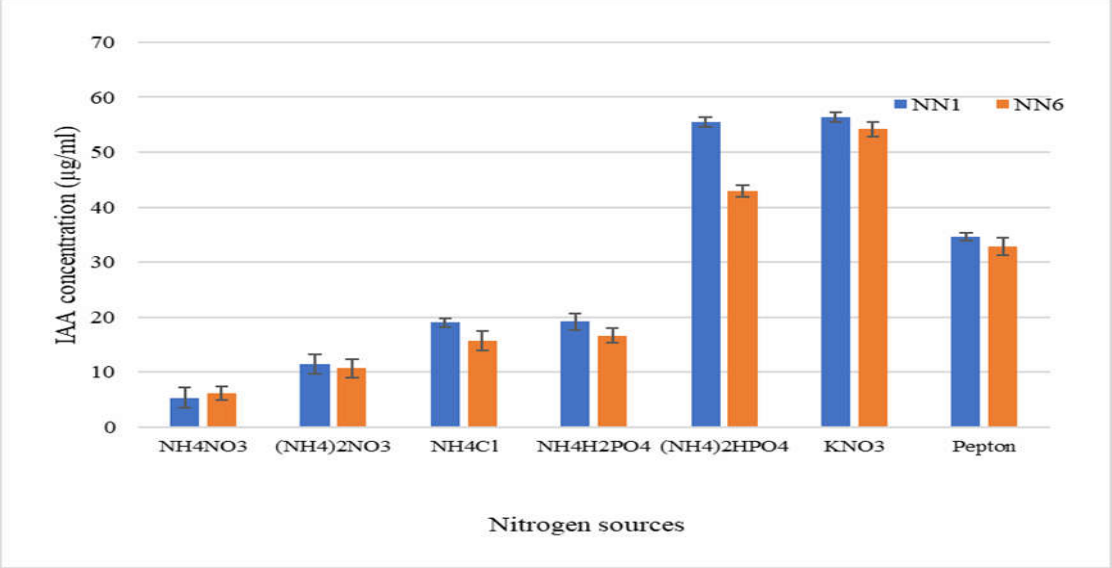


Figure 6. The effect of nitrogen on the IAA production of NN1 and NN6

3.4. Biological characteristics of endophytic bacteria

Table 1. Some biological characteristics of NN1 and NN6

Reaction	NN1	NN6
Gram staining	+	-
Catalase	+	+
Citrate	+	+
Mobility	+	+
MR reaction	-	-
VP reaction	+	+

Some biochemical characteristics of NN1 and NN6 strains were investigated and the main biological characteristics are presented in table 1 and figure 7.

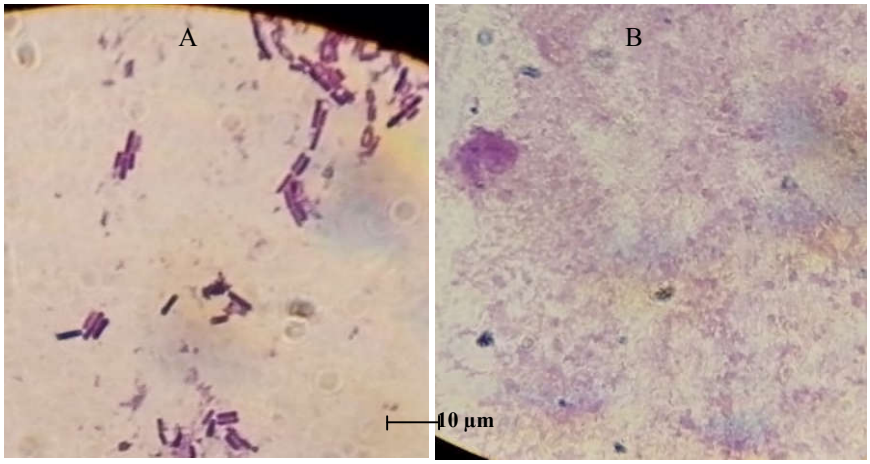


Figure 7. Gram stain results of NN1 (A) and NN6 (B)

4. CONCLUSIONS

From the roots of *P. pseudoginseng,* 16 samples of endophytic bacteria were isolated. All 16 bacteria samples were able to synthesize IAA, of which the samples NN1 and NN6 synthesized the most amount of IAA, with the concentrations at 85.11 µg/mL and 69.29 µg/mL, respectively. NN1 and NN6 bacteria strains produced the highest IAA concentration at 6 days in the nitrogen-contained medium of KNO_3 and (NH_4) HPO_4, pH 6-8. Lastly, dextrin and saccharose are suitable carbon sources for NN1 and NN6 bacteria to synthesize IAA with maximum levels. Cells of endophytic NN1 bacteria samples are bacilli, gram-positive, capable of mobility, cells of endophytic bacteria samples NN6 are bacilli, gram-negative, motile. Both NN1 and NN6 had positive reactions to catalase, VP, citrate reduction, and MR negative reaction. NN1 and NN6 bacteria strains are the potential candidates for the utmost production of IAA in laboratory conditions and these bacteria can be utilized as biofertilizers for promoting plant growth of *P. pseudoginseng*.

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THE MOLECULAR CHARACTER OF *Tacca chantrieri* ANDRÉ IN BA VI NATIONAL PARK BASED ON CHLOROPLAST GENE REGION (*MatK*)

Vu Dinh Giap[1], *, Khuat Thi Minh Hien [2], Vu Dinh Duy[3], Bui Thi Tuyet Xuan[4]

ABSTRACT

Tacca J.R.Forst. & G.Forst. is the only genus in Taccaceae that includes 19 species distributed around the world, of which have found 7 species in Vietnam. DNA barcoding is a rapid species identification and discovery method using short, standardized genes or DNA regions. In this study, we have sequences of chloroplast gene region (*matK*) from 30 *Tacca chantrieri* André collection individuals in Ba Vi National Park to investigate the genetic character and their phylogenetic inference in the genus Tacca. DNA was extracted from the tissue of leaves. The nucleotide sequence of *matK* was determined to be 747 bp and deposited in GenBank (ON337530-ON337559). All samples showed that their length and nucleotide sequence similarity were 100%. The analysis indicated the mean base compositions were nucleotide T(U) (39.9%), C (15.4%), A (29.9%), and G (14.9%). The GC content was found to be low (30.3%), compared to 69.8% of the AT content. Phylogenetic analyses using maximum likelihood (ML) indicated that all samples from Vietnam have a close relationship with *T. chantrieri* in GenBank with strong supporting values (98%). Genetic p-distances interspecific divergence within and among *Tacca* species varied from 0% to 5%, with a mean genetic distance of 2%. The gene (*matK*) can also be used as a DNA barcode to identify the *T. chantrieri* species in Vietnam.

Keywords: *DNA barcodes, matK gene, Tacca chantrieri, phylogenetic tree.*

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1. INTRODUCTION

A total of 19 species of *Tacca* (Taccaceae) have been identified by the World List of Monocotyledonous Plants with 7 species have been recorded in Vietnam, as flow: *Tacca chantrieri, T. integrifolia, T. palmata, T. leontopetaloides, T. plantaginea, T. subflabellata* and *T. khanhhoaensis* [1, 2, 3, 4]. T. chantrieri is a perennial herb that grows 50-80 cm tall. The body is long and has a lot of nodes. Petioles are 10-30 cm long and grow straight from the rhizome, with pointed oval blades 25-60 cm long, 7-20 cm wide, glossy green, wavy borders. The purple-black flowers are clustered on a straight or curved handle 10-15 cm long; the canopy has four purple-brown bracts, the outer bracts oval, pointed, and the inner bracts oval, oblong at the base, with sterile filaments up to 25 cm long. Calyx stalks, 6 stamens, and lower gourds with lateral ovary attachment are all present in the flowers. The fruit does not open on its own; the seeds are crimson purple in hue and have three edges. July-August is the flowering season, while September-October is the fruiting season. This species may be found in the wild in Vietnam's northern regions, where it thrives in streams and damp woodlands. Its rhizomes have been used as folk medicine to treat gastric ulcers, enteritis, and hepatitis [3].

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Phytochemical investigations of this plant have resulted in the isolation of diarylheptanoids [5], and steroidal saponins [6, 7, 8], and sterol saponins [9]. In addition, these compounds show cytotoxic activities [5]. *T. chantrieri* distribution is restricted by overexploitation, habitat destruction, and habitat fragmentation [10].

To identify plant taxon, using a DNA barcode is considered one of the most effective tools available today. Compared to the mitochondrial gene (cytochrome c oxidase I), which has been used effectively to identify many animal groups [11, 12, 13], the synonymous substitution rate of this gene is very low in land plants [14]. Moreover, because of hybridization, polyploidy, and apomixis in plants, identifying DNA barcodes is more difficult. Therefore, the suggestion of using the *trnH-psbA* gene or using a combination of the *trnH-psbA* and rbcL genes as an appropriate tool for plant taxonomy was reported by Kress *et al.* (2005) [15], Kress and Erickson (2007) [16]. In the study of Lahaye *et al.* (2008), the authors analyzed and compared eight gene regions commonly used in plant taxonomy, including *trnH-psbA, matK, ycf5, rbcL, rpoB, ndhJ, accD,* and *rpoC1* [17]. The results showed that the *matK* gene was considered a universal DNA barcode for flowering plants. The *matK* gene can identify cryptic species. However, the application of DNA barcodes to plants has been impeded due to problems such as low variation between species. In a study by the Consortium for the Barcode of Life Plant Working Group (2009), the results recommended that the 2-locus combination of ribulose-1,5-bisphosphate carboxylase (*rbcL*) and maturase (*matK*) as the plant barcode, which can identify and discover overlooked plant species [18]. Currently, different researchers have used some region genes such as *ITS, 18S, matK, psbA-trnH, rbcL, atpA, rbcL, trnL-F,* and *trnS-trnG* in the building of DNA barcodes for the identification of *Tacca* species [10, 19, 20, 21, 22].

In this study, we sequenced the chloroplast gene region (*matK*) to identify the genetic characteristics of *T. chantrieri* and their phylogenetic inference in the genus *Tacca* in Ba Vi National Park (Ba Vi NP). This study contributes to developing a DNA barcode database as a foundation for conservation, evolution, and biological systems.

2. MATERIALS AND METHODS

2.1. Collection sampling

Table 1. Population descriptions of all sampled populations of *T. chantrieri* of Ba Vi NP

Sample code	Locality	Latitude (N)	Longitude (E)	Altitude (m)	GenBank code
BV01	Ba Vi National Park	21°04'57.8"	105°22'007"	747	ON337530
BV02	Ba Vi National Park	21°04'57.8"	105°22'007"	747	ON337531
BV03	Ba Vi National Park	21°04'57.4"	105°22'55"	694	ON337532
BV04	Ba Vi National Park	21°04'56.5"	105°22'6.5"	698	ON337533
BV05	Ba Vi National Park	21°04'56.5"	105°22'6.5"	698	ON337534
BV06	Ba Vi National Park	21°04'55.6"	105°22'8.3"	686	ON337535

BV07	Ba Vi National Park	21°04'57.4"	105°22'31"	687	ON337536
BV08	Ba Vi National Park	21°04'57.4"	105°22'31"	687	ON337537
BV09	Ba Vi National Park	21°03'6.52"	105°21'7.9"	1081	ON337538
BV10	Ba Vi National Park	21°03'6.52"	105°21'7.9"	1081	ON337539
BV11	Ba Vi National Park	21°03'6.52"	105°21'7.9"	1081	ON337540
BV12	Ba Vi National Park	21°03'6.52"	105°21'7.9"	1081	ON337541
BV13	Ba Vi National Park	21°03'6.52"	105°21'7.9"	1081	ON337542
BV14	Ba Vi National Park	21°03'6.52"	105°21'7.9"	1081	ON337543
BV15	Ba Vi National Park	21°03'6.52"	105°21'7.9"	1081	ON337544
BV16	Ba Vi National Park	21°04'51.5"	105°22'1"	700	ON337545
BV17	Ba Vi National Park	21°06'57"	105°24'53"	1076	ON337546
BV18	Ba Vi National Park	21°06'57"	105°24'53"	942	ON337547
BV19	Ba Vi National Park	21°05'15.8"	105°22'58"	942	ON337548
BV20	Ba Vi National Park	21°04'9.40"	105°22'52"	942	ON337549
BV21	Ba Vi National Park	21°04'9.61"	105°22'44"	993	ON337550
BV22	Ba Vi National Park	21°05'6.4"	105°22'56"	993	ON337551
BV23	Ba Vi National Park	21°03'6.37"	105°21'7.8"	993	ON337552
BV24	Ba Vi National Park	21°03'6.27"	105°21'7.6"	993	ON337553
BV25	Ba Vi National Park	21°05'6.15"	105°21'7.4"	997	ON337554
BV26	Ba Vi National Park	21°03'6.04"	105°21'7.1"	819	ON337555
BV27	Ba Vi National Park	21°03'59.3"	105°21'7.1"	985	ON337556
BV28	Ba Vi National Park	21°03'41.3"	105°21'25.8"	985	ON337557
BV29	Ba Vi National Park	21°03'56.6"	105°21'45.7"	972	ON337558
BV30	Ba Vi National Park	21°05'41.1"	105°21'8.56"	774	ON337559

In this study, 30 samples (young leaves) of *T. chantrieri* were collected and placed in plastic bags with silica gel and transferred to the laboratory of Vietnam - Russia Tropical Centre, stored at -30°C for DNA extraction (Table 1).

Figure 1. Adult plant of *T. chantrieri* species collected in Ba Vi NP

[Photo: Dr. Vu Dinh Duy]

2.2. DNA isolation

Total genomic DNA was extracted using a plant/fungi DNA isolation kit (Norgenbiotek, Canada). The total DNA purity and integrity were tested by the Nanodrop ND-2000 spectrophotometer (NanoDrop Technologies, DE, USA) and then diluted to a concentration of 20 ng/µl.

2.3. PCR amplification

The *matK* gene region was amplified through the following PCR cycling profile: an initial heating step at 94°C for 3 min; followed by incubating for 40 cycles of 94°C for 1 min, 55°C for 1 min, respectively, and 72°C for 1 min, and completed by incubating at 72°C for 10 min. All PCR reactions were performed in 25 µl volumes (2.0 µL of template DNA, 12.5 µL of 2X Taq Master Mix, 0.5 µL of each primer, and 9.5 µL of deionized water) using Gene Amp PCR Systems 9700. Double-stranded DNA was directly amplified by symmetric polymerase chain reaction (PCR) amplification using pairs of primers *matK-F* (5'-ACCCTGTTCTGACCGTATCGC-3') and *matK-R* (5'- TCCATTTACATGATCCCATGACC-3') [23, 24].

2.4. Sequencing of the *matK* region

Sequencing was performed on an Avant 3100 automated DNA sequencer using the Dye Terminator Cycle sequencing kit (PE Applied Biosystems). Sequencing of the 30 studied samples used the primers *matK-F* and *matK-R*.

2.5. Phylogenetic analysis

Chromas Pro 2.1.6 software (Technelysium Pty Ltd., Tewantin, Australia) was used to edit the sequences [25]. Sequence alignments were made with Bioedit v7.0.5.2 [26]. We used MEGA 7.0 to analyze our data [27]. Nucleotide sequence divergences were calculated using the Kimura two-parameter (K2P). Phylogenetic trees were performed using maximum likelihood (ML) on MEGA 7.0 software with 1000 replicates. Mega 7.0 was used to analyze p-distance between *Tacca* species.

3. RESULTS AND DISCUSSION

3.1. DNA extraction and polymerase chain reaction

In plants, secondary metabolites and polysaccharides interfere with genomic isolation procedures and downstream reactions such as restriction enzyme analysis and gene amplification [28]. DNA isolation represents the basic and probably the most important step in plant genetics and biotechnology. Despite the development of molecular protocols for DNA isolation of plant species, there are still many drawbacks depending on sample composition. To maximize DNA yields and minimize the co-extraction of PCR inhibitors, we used the Plant DNA Isolation Kit for DNA extraction from 30 leaf tissues of *T. chantrieri* in Ba Vi National Park. Results of DNA electrophoresis on 1% agarose gel showed that each sample had single, sharp, and bold bands indicating successful DNA extraction (figure 2). The purity of extracted DNA was excellent, as evident in DNA concentrations A260/A280 ratio ranging from 1.732 to 2.0, which also suggested that the preparations were sufficiently free of proteins and polyphenolic/polysaccharide compounds. The DNA concentration ranged from 640 to 980 ng/µl. The extracted DNA was suitable for PCR amplification of plant barcode genes. The DNA concentration was diluted for the PCR reaction to 20 ng/µl. The primer pair *matK-F/matK-R* was successfully cloned for 30 samples at a primer temperature of 55°C (Figure 3). The PCR product was approximately 800 bp in length. Electrophoresis on a 1.5% agarose gel showed a high-quality PCR product, with only a single bright band qualified for nucleotide sequencing.

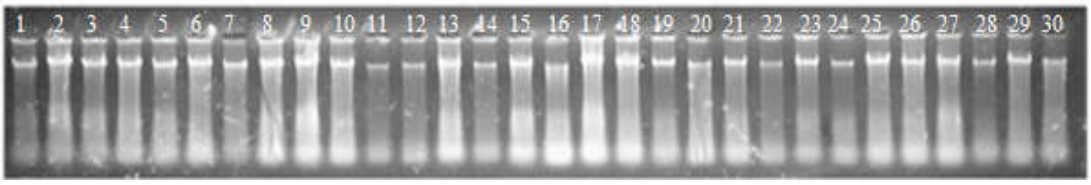

Figure 2. Electrophoresis of total DNA from 30 samples of *T. chantrieri* using 1% agarose gel

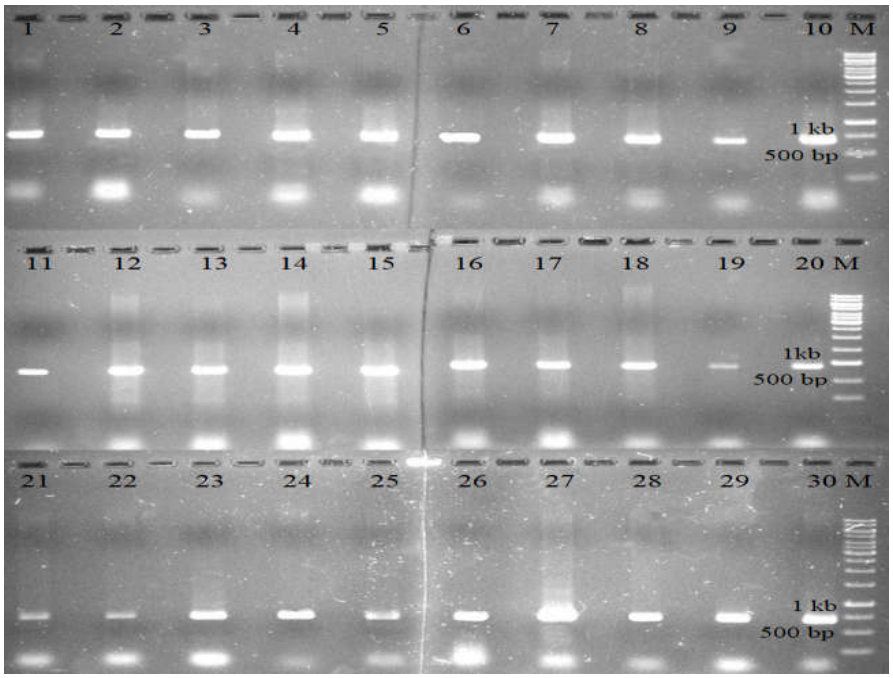

Figure 3. PCR products from 30 samples of *T. chantrieri* were electrophoresed on 1.5% agarose gel (M: DNA ladder 100 bp; 1-30: No. samples)

3.2. Characteristic analysis of *matK* sequences

All the studied samples were successfully amplified for *matK* gene regions with a high sequencing rate of 100%. Chromas Pro2.1.6 software was used to display the results and edit the sequences [25]. After removing the two ends, we identified that the remaining size of each sample was 747 nucleotides. These sequences have been compared with similar sequences on GenBank using the BLAST tool. Results showed that the studied species was 100% similar to *T. chantrieri* (MK153234). The sequences for the *matK* gene region from *T. chantrieri* in Ba Vi NP have been deposited in GenBank (Table 1). Moreover, the results of comparing nucleotide sequences with each other using Bioedit software between nine samples collected at three locations in the *ITS-rDNA* gene region showed that their length and nucleotide sequence similarity were 100%. Therefore, the following study took only one representative sample of this species in Vietnam.

For this study, *matK* sequences of *T. chantrieri* consisted of 747 nucleotide positions. The mean base compositions were 39.9, 15.4, 29.9, and 14.9% for T (U), C, A, and G, respectively. The GC content was low (30.3%) compared to 69.8% of the AT content. This difference showed a low TA content at all three codon positions. These values were 63.8%, 67%, and 78.3% for the 1st, 2nd and 3rd codons, respectively (Table 2).

Table 2. Nucleotide base compositions (%) for the *matK* sequences of *T. chantrieri*

	Codon position	Base				Length (bp)
		T(U)	C	A	G	
Tacca chantrieri	All positions	39.9	15.4	29.9	14.9	747
	1st positions	36.5	20,5	27.3	15.7	249
	2nd positions	34.9	16.9	32.1	16.1	249
	3rd positions	48.2	8.8	30.1	12.9	249

3.3. The genetic distance and phylogenetic inference in the genus *Tacca*

The genetic distances and the maximum likelihood (ML) tree were used to determine genetic relationships between samples (BV) and 15 species of the genus *Tacca* in the GenBank (Table 3 and Figure 4). The mean genetic distance was 2% ranging from BV/*T. chantrieri* (0%) to *T. plantaginea/T. artocarpifolia* (5%). The species pairs: *T. plantaginea/T. artocarpifolia* (5%) showed the highest genetic distances, whereas the lowest genetic distances were observed between species pairs: BV/*T. chantrieri* (0%), *T. leontopetaloides/T. maculate* (0%), *T. havilandii/T. cristata* (1%), *T. reducta/T. cristata* (2%). Our results showed the low genetic distances among species in the genus *Tacca*.

The maximum likelihood tree of sequence divergences (K2P) in the *matK* region reflects the above findings. It shows that all 15 species in the genus *Tacca* were distinctly separated and characterized by a high bootstrap value and a branch length of 0.005 (Figure 4). The ML tree showed a clear separation of samples (BV) into one clade together with *T. chantrieri* (MK153234) with a bootstrap value of 98%. BV/*T. chantrieri* had

highly identical *matK* sequences. The results showed that this species pair was determined as one species.

Table 3. Different genetic distances among species in genus *Tacca* based on *matK* analysis

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1. BV	-															
2. *T. chantrieri* MK153234	0.00	-														
3. *T. subflabellata* JF956654	0.02	0.01	-													
4. *T.plantaginea* JF956647	0.03	0.03	0.04	-												
5. *T. leontopetaloides* JF956642	0.03	0.03	0.03	0.04	-											
6. *T. ampliplacenta* JF956614	0.02	0.02	0.01	0.04	0.04	-										
7. *T. integrifolia* JF956637	0.01	0.01	0.01	0.03	0.03	0.00	-									
8. *T. havilandii* MK153235	0.01	0.01	0.01	0.03	0.03	0.01	0.01	-								
9. *T. borneensis* MK153231	0.01	0.01	0.01	0.03	0.03	0.01	0.01	0.01	-							
10. *T. bibracteata* MK153225	0.02	0.02	0.02	0.03	0.03	0.02	0.02	0.02	0.02	-						
11. *T. sumatrana* MK153224	0.01	0.01	0.02	0.03	0.03	0.02	0.01	0.01	0.01	0.02	-					
12. *T. cristata* MK153223	0.01	0.01	0.01	0.03	0.03	0.01	0.01	0.00	0.01	0.02	0.01	-				

13. *T. reducta* MK153216	0.01	0.01	0.02	0.03	0.03	0.02	0.01	0.01	0.01	0.02	0.00	0.01	-			
14. *T. palmata* MK153217	0.02	0.02	0.03	0.03	0.03	0.03	0.02	0.02	0.02	0.02	0.02	0.02	0.02	-		
15. *T. maculata* MK153197	0.03	0.03	0.04	0.04	0.01	0.04	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	-	
16. *T. artocarpifolia* KU308827	0.03	0.03	0.04	0.05	0.01	0.04	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.01	-

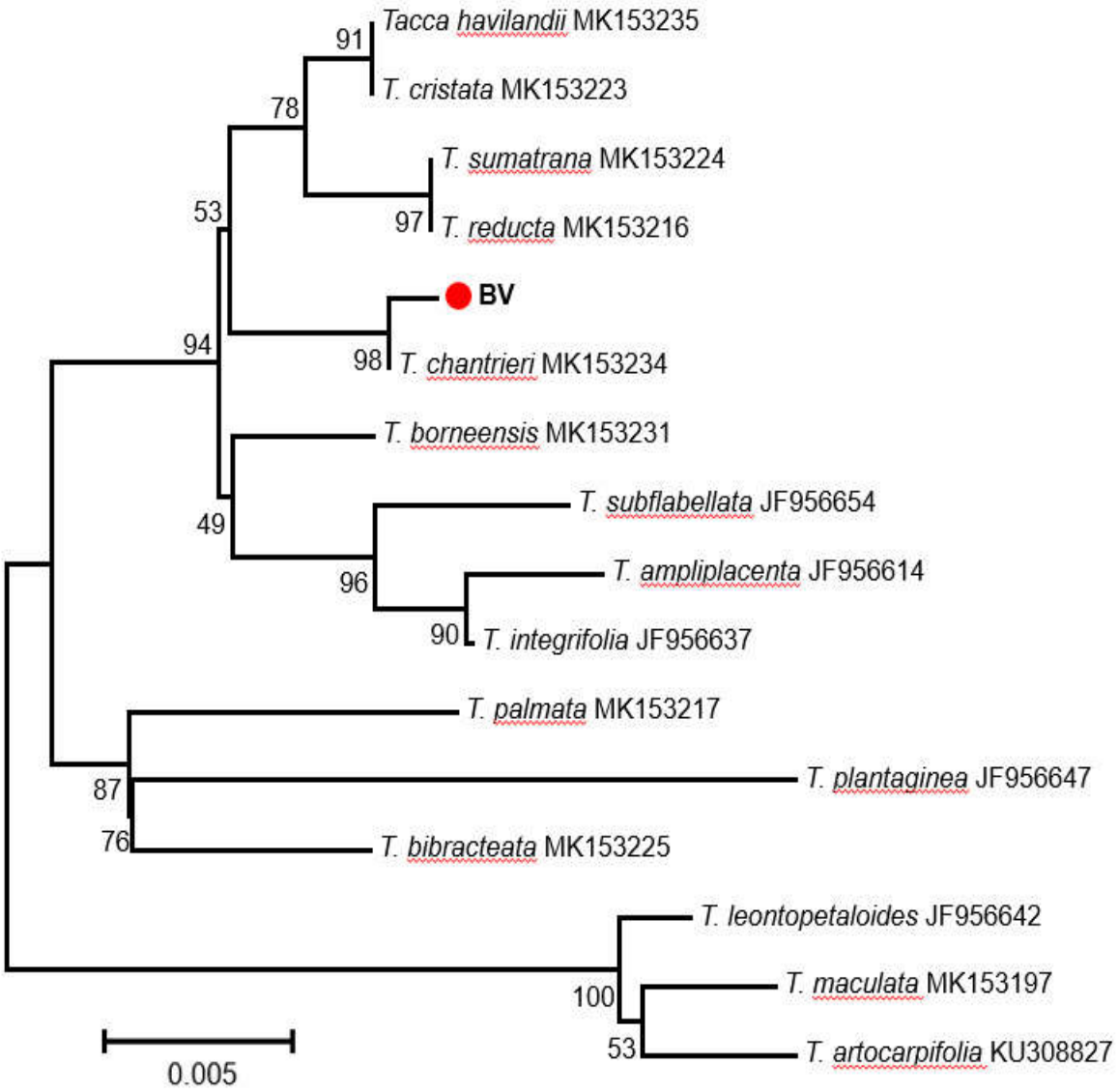

Figure 4. Phylogenetic relationships among *Tacca* species based on the sequence of chloroplast gene (*matK*) using the Maximum likelihood (ML) tree. The numbers above branches represent bootstrap

Accurate species identification is essential for managing and conserving species [29]. Species identification based on morphology is mostly uphill and less accurate. However, molecular-based methods such as DNA barcoding are rapid and accurate for specific identification [30, 31, 32]. A previous study by Zhao and Zhang (2011) recommended four candidate DNA barcoding regions, three (*rbcL, matK,* and *trnH-psbA*) from the chloroplast genome and one (*ITS*) from the nuclear genome, which was evaluated among 36 accessions representing 6 species of *Tacca* [33]. The results indicated that both *ITS* and the core barcode *rbcL+matK* proposed by the Consortium for the Barcode of Life (CBOL) exhibited the highest resolution as single and combined data, respectively. Based on overall performance, *matK+rbcL* can be considered a potential barcode for identifying the species of *Tacca*. *ITS* can be used as a supplementary barcode. DNA barcoding revealed two distinct lineages of *T. integrifolia* distributed allopatrically in Tibet and Malaysia. And these two lineages with morphological variations may potentially represent new species. Zhang *et al.* (2011) used DNA sequences from one nuclear, one mitochondrial, and three plastid loci (*ITS, atpA, rbcL, trnL-F,* and *trnH-psbA*) to reconstruct molecular phylogeny in the genus *Tacca* [10]. Phylogenetic analysis of 16 *Tacca* species utilizing nuclear *ITS* and plastid *matK* gene areas [21]. Our research indicates that the genetic distance between the species *Tacca* (2% *matK* gene) is less than that between nuclear gene regions (10% *ITS-rDNA gene*) [22]. Our results agree with the previous finding, confirm the core barcode's effectiveness, and suggest using *matK + ITS-rDNA* gene region as DNA barcode sequences in the genus *Tacca* in Vietnam

4. CONCLUSIONS

In the current study, we sequenced chloroplast gene region nucleotides (*matK*) to identify *T. chantrieri* in Vietnam, constructed phylogenetic trees of the genus *Tacca*, and suggested using *matK* gene region to identify *Tacca* species in Ba Vi NP. The findings there will be significant in the study of evolution, systematics, and conservation of the species.

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USE OF PINEAPPLE CORE AND DURUM SEMOLINA IN PASTA MAKING: EFFECTS OF DIFFERENT RATIOS OF PINEAPPLE CORE POWDER ON THE PRODUCT QUALITY

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ABSTRACT

Pineapple core is a by-product of canned sliced pineapple in syrup. In this study, this by-product was proved to be a source of dietary fiber and phenolic compounds with high antioxidant activity. Pineapple core powder (PCP) was then added to pasta recipe at 0 (control), 5, 10, 15 and 20% of the blend weight for pasta making. The effects of different ratios of PCP on the pasta quality were investigated. Increase in PCP ratio improved total dietary fiber, and phenolic content as well as antioxidant activity of the product but reduced its starch content. In addition, high level of PCP in the pasta formulation resulted in high hardness and cooking loss but low elongation rate, tensile strength, and overall acceptability. When the PCP ratio varied from 5 to 15%, the obtained pasta was high fiber food with acceptable sensory quality. PCP is a potential ingredient in the formulation of pasta with high fiber and antioxidant content.

Keywords: *Antioxidant, dietary fiber, pasta, pineapple core.*

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1. INTRODUCTION

Dietary fiber and antioxidants are noticeably crucial supplements for human diet since they can make positive impacts on health such as improving digestive system, reducing blood cholesterol, preventing obesity, type-2 diabete, and colonic cancer [1]. Pasta is a well-known food worldwide and the consumption of pasta has been increasing in the world market. However, traditional pasta is poor in dietary fiber and antioxidant [2]. From the last recent years, different by-products originated from food processing have been added to pasta recipe for the improvement in dietary fiber and antioxidant content such as cellulase-treated wheat bran [3], apple pomace [4], grape pomaces [5]. Nevertheless, high ratio of food by-products in the pasta formulation decreases textural and cooking properties as well as sensory quality of the product [3-5]. It should be noted that the use of by-products in food industry to make pasta with high dietary fiber and antioxidant content is a sustainable development for a better exploitation of agricultural products.

Pineapple is tropical fruit which has been widely cultivated in Vietnam. This fruit is industrially processed into different food products, among which canned pineapple slices in syrup is a popular canned food due to high convenience, long shelf-life, high microbiological quality. In the production of canned pineapple slices in syrup,

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pineapple core is a by-product which is conventionally used for animal feeding. It is reported that pineapple core is treated and utilized as a dietary fiber source for supplementation in bakery products such as doughnut and steamed bread [6]. However, the use of pineapple core in pasta making has not been considered in the world.

In this research, pineapple core powder (PCP) was added to durum semolina for pasta making. The aim of this study was to investigate the impacts of different ratios of PCP on the pasta quality including proximate composition, antioxidant activity, cooking quality, texture profile and overall acceptability.

2. MATERIALS AND METHODS

2.1. Materials

Pineapple (*Ananas comosus* L.) core was provided by Viet Duc Food Technology Co., Ltd. Durum semolina was purchased from Vietnam Wheat Milling Co., Ltd. Refined salt with added iodine was supplied by Southern Salt Group, Vietnam.

2.2. Analytical chemicals

Enzyme preparations including α-amylase Termamyl®SC, glucoamylase Dextrozyme®GA and protease Neutrase®2.5L used for dietary fiber determination were provided by Novozymes (Denmark). Chemicals with analytical grade were originated from Sigma-Aldrich (USA) and Xilong Scientific Company (China).

2.3. Preparation of pineapple core powder (PCP)

Fresh pineapple core was milled and pressed for juice extraction. The resulting pomace of pineapple core was sterilized at 115°C for 10 min to inactivate microorganisms and enzymes, then dried at 50°C for 4 h until the moisture content reached 13%. The dried product was sieved through a 70 mesh (210 µm) sieve and the product powder was stored in the freezer at 4°C for further experimentation.

2.4. Pasta making procedure

In this recipe, the blend of durum semolina and PCP with total weight of 150 g and 0,75 g salt were dry-mixed at room temperature in a mixer at 72 rpm for 3 min to form a uniform mixture. The dry mix was then added with 68 g water at 42°C and kneaded in 20 min using a stand mixer to produce a uniform dough. The obtained dough was extruded by an extruder to make pasta. The pasta was subsequently dried at 50°C for 8 h until it reached 9-11% moisture content. The ratio of PCP was 0% (control), 5%, 10%, 15% and 20% of the blend weight.

2.4. Analytical methods

Moisture content was measured by drying to constant weight using an infrared moisture analyzer. Total protein was determined by Kjeldhal-Nessler method (AOAC 984.13). Lipid was quantified followed Soxhlet method (AOAC 960.39). Ash was determined according to AOAC 9930.30 method. Starch was measured following AOAC 996.11 method. Total, insoluble and soluble dietary fiber (TDF, IDF and SDF) was estimated according to AOAC 985.29, 991.42 and 991.43 method, respectively. Total phenolics were determined by colorimetric method with Folin-Ciocalteu reagent. Antioxidant activity including DPPH (2.2-diphenyl-1-picrylhydrazyl) radical scavenging activity and ferric reducing power (FRAP) were analyzed according to the procedure described by Nguyen *et al.* (2020) [3]. The particle size distribution of PCP and durum semolina samples was determined by a laser diffraction particle size distribution analyzer (Horiba, Japan); the mean particle size (d4,3) and specific surface area were calculated using LA-920 software (Horiba, Japan); the span value was calculated by the formula reported by Kippax (2005) [7]. Water and oil holding capacity of wheat semolina and

PCP as well as optimal cooking time, cooking loss, and swelling index of pasta samples were measured following the procedure reported by Nguyen *et al.* (2020) [3]. Instrumental color data including L*, a*, b* indexes were quantified by a CM-3700A colorimeter (Konica Minolta, Japan); the color difference (ΔE) between the control pasta and the pasta added with PCP was calculated by the formula described by Martin (2015) [8]. Textural properties of cooking pasta samples were analyzed by a TA-TX structural analyzer (Stable Micro Systems Co., UK) coupled with the Exponent Connect Lite 7.0 software. Overall acceptability of pasta samples was evaluated using a nine-point hedonic test [3]. Sixty untrained panelists were randomly selected from the students at Ho Chi Minh city University of Technology (HCMUT) without training and gender consideration. About 1 L of water was used to cook every 100 g of raw pasta according to their optimal cooking time. Then, 30 g of each cooked samples were labeled with different codes and served at the same time with randomized order to each panelist. Water was also provided for mouth cleansing between samples. The panelists were asked to consume the cooked pasta and rate their overall acceptability ranging from 1 (extremely dislike) to 9 (extremely like).

2.5. Statistical analysis

All experiments were repeated three times to calculate the average value. The results are presented as mean ± standard deviation. The experimental results were processed by Analysis of Variance (ANOVA) using the Statgraphics Centurion XVI software (USA). Significant differences were ultimately determined by multiple range test ($p < 0.05$).

3. RESULTS AND DISCUSSION

3.1. Proximate composition, antioxidant activity, and physical properties of pineapple core powder and durum semolina

Table 1 shows the proximate composition, antioxidant activity, and physical properties of PCP and durum wheat semolina. The moisture content of durum semolina was slightly higher than that of PCP. Additionally, the protein and starch content of durum semolina were significantly higher than that of PCP. In contrast, the lipid and ash content in PCP was 1.9 and 3.6 times, respectively, higher than that of durum semolina. The dietary fiber content is an important indicator to evaluate the potential use of PCP as a source of dietary fiber. In this study, the TDF content of PCP was approximately 19 times higher than that of durum semolina. PCP was therefore proved to be a good dietary fiber source for pasta supplementation.

Phenolic compounds are representative antioxidants of PCP [9]. The total phenolic content in PCP was about 2.4 times higher than that in durum semolina. As a result, the DPPH radical scavenging activity and ferric reducing power of PCP were significantly greater than those of durum semolina. Thus, PCP was also a potential antioxidant source for supplementation to different food products.

In terms of physical properties, the particle size mean of PCP was smaller than that of semolina while the specific surface area of PCP was greater. The higher SPAN index of PCP revealed a better uniformity in particle size as compared to that of durum semolina. The water holding capacity of PCP was 7.9 times higher than that of semolina due to its higher fiber content and specific surface area. It should be noted that the hydroxyl group of fiber could interact with water [10], resulting in high water holding capacity for fiber material. Similarly, the oil holding capacity of PCP was 4.3 times higher than that of durum semolina due to porous structure of pineapple core fibers [11].

The instrumental color data showed that durum semolina had slightly higher brightness (L* = 91.2) than PCP (L* = 85.7).

Table 1. Proximate composition, antioxidant activity, and physical properties of pineapple core powder and durum semolina

Raw materials	Pineapple core powder	Durum wheat semolina
Moisture (%, ww)	9.3 ± 0.1 [a]	10.2 ± 0.1 [b]
Crude protein (%, dw)	5.1 ± 0.1 [a]	13.8 ± 0.1 [b]
Lipid (%, dw)	3.1 ± 0.1 [b]	1.6 ± 0.1 [a]
Ash (%, dw)	1.8 ± 0.1 [b]	0.5 ± 0.1 [a]
Starch (%, dw)	3.5 ± 0.1 [a]	80.2 ± 1 [b]
TDF (%, dw)	65.7 ± 0.4 [b]	3.3 ± 0.5 [a]
IDF (%, dw)	2.4 ± 0 [b]	1.4 ± 0.3 [a]
SDF (%, dw)	63.3 ± 0.4 [b]	1.9 ± 0 [a]
Total phenolics (mgGAE/ kg dw)	2554 ± 99 [b]	1051 ± 76 [a]
Ferric reducing power (µmolTE/ kg dw)	5381± 55 [b]	753± 21 [a]
DPPH radical scavenging activity (µmol TE/ kg dw)	4634 ± 98 [b]	1316 ± 74 [a]
Water holding capacity (g water/g dw)	6.3 ± 0.1 [b]	0.8 ± 0.1 [a]
Oil holding capacity (g oil/g dw)	3 ± 0.1 [b]	0,7 ± 0.1 [a]
Mean particle size (µm)	117.7 ± 3.1[a]	182.7 ± 5.5[b]
Specific surface area (m^2 /kg)	738.5 ± 16.7 [b]	277.4 ± 14.3 [a]
SPAN	2.8 ± 0.1 [a]	3 ± 0.1 [b]
L*	91.2 ± 0[b]	85.7 ± 0.1 [a]

Values that do not share a lowercase letter (a-d) within a row are significantly different ($p < 0.05$).

3.2. Proximate composition of pasta

Table 2 presents the proximate composition of pasta with different PCP ratios. The moisture content of all pasta samples was statistically similar ($p>0.05$), and this moisture content met the required standard for pasta storage at room temperature. When the PCP ratio of the pasta formulation increased from 0 to 20%, the protein content of the product reduced by 13% while its lipid content increased by 22%. That is due to the

difference in protein and lipid content of PCP and durum semolina. Nevertheless, the increased PCP ratio did not result in significant change in ash content of the pasta since the difference in ash content between PCP and durum semolina was little.

Increase in PCP ratio in the pasta recipe from 0 to 20% increased the SDF, IDF and TDF content of the product by 1.3; 7.8 and 4.8 times, respectively, but decreased its starch content by 19%. Similar increase in dietary fiber content was also reported when grape pomace was supplemented to the pasta formulation [5]. It should be noted that when the PCP ratio was 5% or higher, the resulting pasta was considered as high fiber food since its TDF content was higher than 6 g/100 g [12].

Table 2. Proximate composition of pasta with different ratios of pineapple core powder

Ratio of pineapple core powder (%)	0	5	10	15	20
Moisture (%, ww)	9.0 ± 0.1^{a}	9.0 ± 0.1^{a}	9.0 ± 0.2^{a}	9.0 ± 0.1^{a}	9.0 ± 0.1^{a}
Crude protein (%, dw)	12.9 ± 0.2^{d}	12.6 ± 0.1^{d}	12.2 ± 0.1^{c}	11.7 ± 0.1^{b}	11.2 ± 0.2^{a}
Lipid (%, dw)	1.8 ± 0.2^{a}	1.9 ± 0.2^{a}	2.0 ± 0.1^{ab}	2.1 ± 0.1^{b}	2.2 ± 0.1^{b}
Ash (%, dw)	0.8 ± 0.1^{a}	0.8 ± 0^{a}	0.8 ± 0.1^{a}	0.9 ± 0.1^{a}	0.9 ± 0.1^{a}
Starch (%, dw)	80.2 ± 0.7^{e}	76.4 ± 0.4^{d}	72.5 ± 0.6^{c}	68.7 ± 0.3^{b}	64.9 ± 0.5^{a}
TDF (%, dw)	3.3 ± 0.1^{a}	6.3 ± 0.2^{b}	10.1 ± 0.3^{c}	12.8 ± 0.3^{d}	15.8 ± 0^{e}
IDF (%, dw)	1.8 ± 0.1^{a}	4.8 ± 0.1^{b}	8.5 ± 0.3^{c}	11.1 ± 0.3^{d}	14.0 ± 0^{e}
SDF (%, dw)	1.4 ± 0^{a}	1.5 ± 0^{b}	1.6 ± 0.1^{b}	1.7 ± 0^{c}	1.8 ± 0^{d}

Values that do not share a lowercase letter (a-d) within a row are significantly different ($p < 0.05$)

3.3. Antioxidant activity of pasta

Figure 1 demonstrates the effects of PCP supplementation on the phenolic content and antioxidant activity of pasta. When the PCP ratio was enhanced from 0 to 20%, the total phenolic content of the pasta increased by 1.2 times while its DPPH radical scavenging activity and ferric reducing power were improved by 1.7 and 2.9 times, respectively. Similar results were also recorded in the study of Yadav and Gupta (2015) when apple pomace was added to pasta formulation [4]. Thus, PCP was a good source for both fiber and antioxidant supplementation to pasta products.

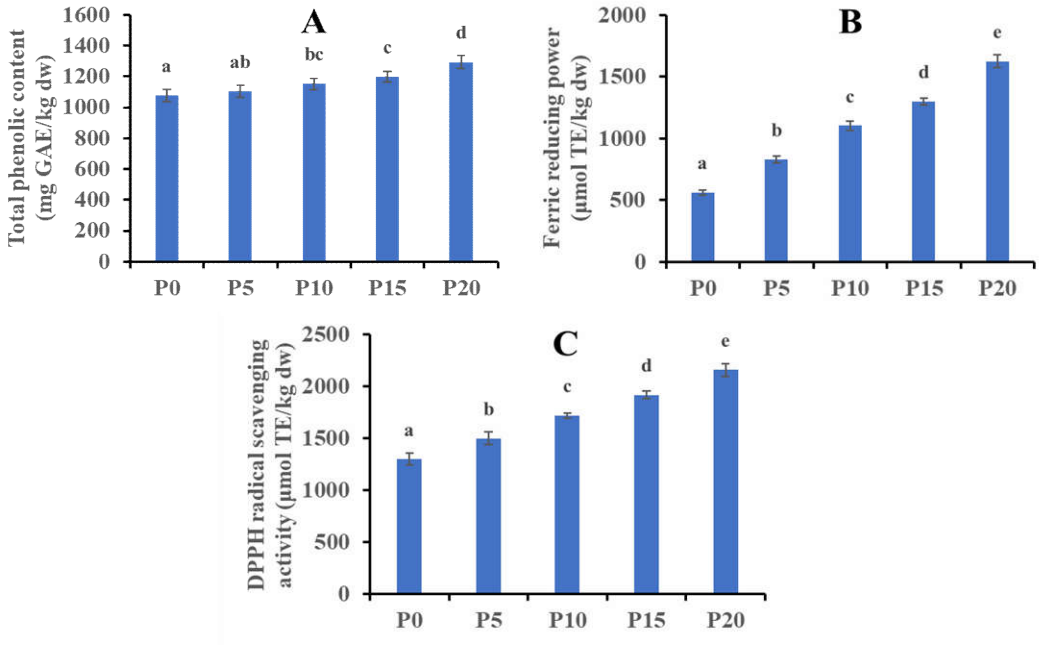

Figure 1. Total phenolic content (A), ferric reducing power (B), and DPPH radical scavenging activity of pasta with different pineapple core powder ratios

Values that do not share a lowercase letter (a-d) within each sub-figure are significantly different ($p < 0.05$)

3.4. Cooking quality of pasta

Table 3 illustrates the effects of PCP addition on the cooking quality of pasta. Increase in PCP ratio from 0 to 20% decreased the optimal cooking time by 26%. On the other hand, when the level of PCP increased from 0 to 20%, the cooking loss was enhanced by 64%. It can be explained that the use of PCP decreased gluten content in the pasta dough as well as resulted in the distribution of fiber molecules in gluten-starch network of the dough [5]. These modifications could cause a physical disturbance of the gluten-starch network, allowing water rapidly to diffuse into pasta as well as dissolved compounds of pasta to release more into the cooking water. When durum bran and germ (pollard) were added to pasta formulation, the reduction in optimal cooking time and increase in cooking loss were also reported [13].

Besides, the supplementation of PCP to pasta recipe also decreased the swelling index of the product. At 20% PCP level, the swelling index decreased by 39% compared to that of the control probably due to a reduction in starch content of pasta. During pasta cooking, water adsorption was observed for starch granules which are mainly responsible for pasta swelling [14].

Table 3. Cooking quality of pasta with different pineapple core powder ratios

Ratio of pineapple core powder (%)	0	5	10	15	20
Optimal cooking time (min)	13.4 ± 0.1^{e}	12.3 ± 0.2^{d}	11.8 ± 0.2^{c}	10.8 ± 0.2^{b}	9.9 ± 0.1^{a}
Cooking loss (%)	4.5 ± 0.1^{a}	4.8 ± 0.1^{ab}	5.4 ± 0.1^{b}	6.5 ± 0.4^{c}	7.4 ± 0.7^{d}
Swelling index	1.8 ± 0.1^{d}	1.8 ± 0.1^{d}	1.5 ± 0.1^{c}	1.3 ± 0.1^{b}	1.1 ± 0.1^{a}

Values that do not share a lowercase letter (a-d) within a row are significantly different ($p<0.05$).

3.5. Instrumental color, textural properties, and overall acceptability of pasta

The effects of PCP use in pasta recipe on the instrumental color, texture profile, and overall acceptability of pasta are visualized in table 4. The increased PCP level in the recipe slightly decreased the L* value but enhanced both a* and b* values. In practice, the pasta samples with PCP were slightly darker, and their yellow color became more obvious. Nevertheless, the color difference between the PCP added pasta and the control was little since the ΔE value varied from 0 to 5.9.

The use of PCP enhanced the hardness of pasta. At 20% PCP level, the hardness of pasta was 64% higher than that of the control. The increased fiber content might reduce the water absorption and swelling capacity of the starch granules, leading to the increased hardness of pasta [14]. In contrast, the cohesiveness decreased by 12% for the pasta sample with 20% PCP level as compared to that of the control. For high fiber pasta, the network between protein and starch molecules was disrupted by fiber compounds, leading to an increase in cooking loss and a reduction of cohesiveness [3]. The gumminess and chewiness of pasta sample supplemented with 20% PCP level were 44% and 41%, respectively, higher than those of the control. The same tendency was recently observed in the study of Sohaimy *et al.* (2020) when durum semolina and chickpea powder were used in pasta making [15]. Furthermore, the addition of PCP to pasta recipe decreased tensile strength and elongation rate since the presence of fibers might disrupt gluten network of the product [3].

Table 4 also reveals that the overall acceptability of pasta gradually decreased with the increased proportion of PCP. However, the pasta samples with PCP level of 5, 10 and 15% were considered acceptable since their overall acceptance score was above 5 points. The recommended ratio of PCP used in making fiber with high dietary fiber and antioxidant content was therefore from 5 to 15% of the blend weight.

Table 4. Color values, texture profile and overall acceptability of pasta

Ratio of pineapple core powder (%)	0	5	10	15	20
L*	90.4 ± 0.1^{e}	89.5 ± 0^{d}	88.5 ± 0.1^{c}	87.3 ± 0^{b}	86.2 ± 0.1^{a}
a*	0.9 ± 0^{b}	0.8 ± 0^{a}	0.9 ± 0^{b}	1.1 ± 0^{c}	1.3 ± 0^{d}
b*	8.8 ± 0.2^{a}	10.0 ± 0.2^{b}	11.3 ± 0.2^{c}	12.3 ± 0.4^{d}	12.9 ± 0.4^{e}
ΔE	0 ± 0^{a}	1.5 ± 0.2^{b}	3.1 ± 0.2^{c}	4.7 ± 0.3^{d}	5.9 ± 0.3^{e}
Hardness (g)	2321 ± 59^{a}	2956 ± 60^{b}	3274 ± 78^{c}	3545 ± 92^{d}	3814 ± 82^{e}
Gumminess (g)	1510 ± 26^{a}	1806 ± 57^{b}	1932 ± 49^{c}	2050 ± 62^{d}	2171 ± 98^{e}

Chewiness (g)	1465 ± 28 [a]	1745 ± 53 [b]	1854 ± 63 [c]	1959 ± 82 [d]	2063 ± 79 [e]
Cohesiveness	0.65 ± 0.01 [d]	0.61 ± 0.01 [c]	0.59 ± 0.01 [b]	0.58 ± 0.01 [ab]	0.57 ± 0.02 [a]
Tensile strength (kPa)	32 ± 0.9 [e]	29.1 ± 0.6 [d]	26.6 ± 0.5 [c]	23.9 ± 1.1 [b]	21 ± 0.9 [a]
Elongation rate (%)	97.1 ± 4.8 [e]	48.7 ± 2 [d]	36.9 ± 1.7 [c]	31.1 ± 1.5 [b]	16.4 ± 0.7 [a]
The overall acceptability	6.8 ± 1 [d]	6 ± 1.1 [c]	5.4 ± 0.9 [b]	5.3 ± 0.9 [b]	4.4 ± 0.9 [a]

Values that do not share a lowercase letter (a-d) within a row are significantly different (Turkey's comparison test, $p < 0.05$)

4. CONCLUSION

PCP was proved to be a good source of fiber and phenolic compounds with considerably higher antioxidant activity than durum semolina. When the PCP ratio in pasta recipe increased, the dietary fiber and total phenolic content as well as the antioxidant activity of the pasta were significantly improved while the starch content decreased. In addition, increase in PCP ratio in the pasta formulation enhanced hardness of the product but reduced its elongation rate and tensile strength. Pasta with increased PCP ratio had enhanced cooking loss but its optimal cooking time, swelling index and overall acceptability decreased. When the PCP ratio varied from 5 to 15%, the obtained pasta was a high fiber product and accepted by consumers. Therefore, businesses can consider the addition PCP to pasta formulation in the range of 5 to 15% depending on target consumers. Use of PCP for pasta fortification with dietary fiber and antioxidant was potential for development of healthy food products.

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A NEWLY EMERGING INSECT PEST DAMAGING *Cinnamomum cassia* PLANTATIONS IN YEN BAI PROVINCE

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ABSTRACT

Plantations of *Cinnamomum cassia* are important contributors to the livelihoods of communities living in the mountainous areas of northwest Vietnam. Recently, a *Cricula* species (Lepidoptera: Saturniidae) has emerged as a major threat to these plantations. Morphologically the species is similar to *Cricula variabilis* Naumann & Loffler in China, but preliminary analysis of the mitochondrial cytochrome oxidase 1 gene region indicates the need for detailed phylogenetic analysis to confirm the taxon in Vietnam. In a laboratory study (30±2°C, 85±5% RH and 12 h light: 12 h dark photoperiod), the life cycle took 69.6±5.2 days, females laid 200 to 310 eggs, eggs hatched after 8 to11 days, the larval period was 38 to 48 days, the pupal period was 9 to 15 days, and adults survived 4 to 8 days. There are four generations per year in the field. In ten plantations in Yen Bai province assessed in 2019, the damage incidence ranged from 47.2 to 71.5% and the damage index from 1.03 to 2.14. Cinnamon growers are concerned that the severity of defoliation will lead to a loss in bark yield and reduced income. Field studies are underway to develop an integrated pest management plan for this pest.

Keywords: *Cinnamomum cassia, Cricula variabilis, folivore, life cycle.*

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1. INTRODUCTION

Cinnamomum cassia (Lauraceae) is an evergreen tree, native to southern China and is widely cultivated in South and Southeast Asia [1] mainly for its bark. It has been cultivated commercially in Vietnam since the late 18th Century. The bark is removed for extracting essential oil when trees are 10-12 years old, but the best oil content occurs in older trees. The bark has high economic value due to its oil content [2], [1]; and is popular for seasoning food and processing into pharmaceutical products [2], [1], [3], [4]. In Vietnam, *C. cassia* has been planted in 4 ecological regions: the northwest, northeast, north central and south central. By the end of 2020, the area of *C. cassia* plantations in Yen Bai province in northwest Vietnam was about 78,000 ha, accounting for two-thirds of the total area of *C. cassia* plantations in Vietnam. Most of the *C. cassia* plantations in this area are owned by local people, with each household having about 5-10 hectares.

More than 70 insect pest species have been recorded damaging *C. cassia* [5] and the most common pests in Vietnam are species of *Aegeria*, *Biston, Sophophora* and *Phyllocnistis* genus [6], [7], and *Aetherastis grandisalba* [8], [9], [10], [7]. Recently, a species of *Cricula* (Lepidoptera: Saturniidae) has become a pest of *C. cassia* plantations in Vietnam. According to unpublished data of the Yen Bai's Department of Agriculture

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and Rural Development, this leaf eating pest was first observed causing damage to 262 hectares of *C. cassia* in Yen Bai province in 2016. Since then, local records reveal that damage has increased over time and expanded to Lao Cai and other provinces in northwest Vietnam. The pest in Vietnam has been tentatively assigned to *Cricula variabilis* which was first described in central China in 2010 [11]. In China, the larvae damage a range of trees within the Lauraceae and Fagaceae, including *Callicarpa kochiana, Castanopsis fissa, Cinnamomum burmanni, C. camphora, Machilus chinensis, Mucuna pruriens, Phoebe bournei* and *Schefflera octophylla* [12], [19].

To date, no formal studies have been undertaken on the biology of the *Cricula* damaging commercial stands of *C. cassia* in Vietnam. The purpose of this paper is to identify the taxon, describe the life cycle and morphological characteristics of *C. variabilis*, and to document the damage to *C. cassia* plantations. This information will help alert the forestry and biosecurity sectors to this new emerging pest.

2. MATERIALS AND METHODS

2.1. Insect rearing in the laboratory

Adults were obtained from pupae of *Cricula* collected from *C. cassia* plantations in Tran Yen district, Yen Bai province in 2019. The pupae were separated in Petri dishes (9 x 1.5 cm), and taken to the laboratory in Ha Noi and maintained at 30±2 (SE)°C, 85±5% RH and 12 h light: 12 h dark photoperiod. In order to obtain fertilized eggs, ten pairs of newly emerged adults were released into insect cages 0.6 x 0.6 x 1.0 m (length x width x height). Each cage contained fresh leaves of *C. cassia* trees for laying eggs. Cotton wool inserted into a container of 5% honey solution was placed in each cage to provide food for adults. After hatching, 100 newly emerged larvae were placed in insect cages 40 x 40 x 40 cm (length x width x height) with fresh leaves and kept in acclimatized chambers as for the pupae. The cages were inspected every day to replace the leaves and to record development and mortality of each instar.

2.2. Identification and description

Identification was based on the external morphology of 60 adult specimens (30 males and 30 females) and male genital characteristics, using the keys in Naumann and Löffler (2010) [11]. All specimens were reared in the laboratory. Fourteen male adult and twenty female adult specimens were used for description. A further 90 samples (30 eggs, 30 larvae and 30 pupae) were used to take measurements. The egg size and the length of the larvae were measured using a Leica M165C microscope (Leica Microsystems, Wetzlar, Germany). Images were captured using a Nikon DS-Fi2 camera (Nikon Cooperation, Ha Noi, Vietnam). Adult specimens examined were deposited in the insect collection of the Forest Protection Research Centre (FPRC) with numbers from M001 to M060.

Bodies of three males (M010-M012) were softened in boiled water for 1-2 hours. The adult genitalia were then detached from the abdomen with fine forceps. The genitalia were pretreated in a warm solution of 10% KOH for 10-20 minutes, washed in water several times, and then transferred into glycerol on microscope slides. Microscope images were obtained as described earlier and the structures were compared with the descriptions of Naumann and Löffler (2010) [11].

For molecular identification, the mitochondrial cytochrome oxidase 1 (CO1) gene region was sequenced using mtDNA extracted from legs of male and female adult specimens M001 (21°73'64"N 104°80'94"E) and M002 (21°73'64"N 104°80'94"E) respectively, using both forward and reversed primers COI-LEP-F (ATTCAACCAATCATAAAGATA TTGG) and COI-LEP-R (TAAACTTCTGGATGTCC AAAAAATCA) [14]. The detailed procedures have been described previously in Quang *et al.* (2021) [15]. The DNA sequences of M001 and M002 were

edited by Geneious version 7.1.9 sofware (Biomatters Ltd.) and compared with the database on BOLDSYSTEMS via identification tool (https://v3.boldsystems.org/). The sequences of M001 and M002 were aligned and compared for the similarity with referenced sequences obtained from BOLDSYSTEMS via Geneious version 7.1.9 sofware.

2.3. Assessment of damage and severity

According to surveys undertaken by the Forest Protection Research Centre and Yen Bai's Department of Agriculture and Rural Development (unpublished data), *C. variabilis* in Vietnam has four generations per year and the second generation has the highest larval density and causes damage mainly in April and May. Therefore, six field surveys were carried out in April and May 2019 in 4 to 13-year-old pure plantations of *C. cassia* in Yen Bai province. Six plots (25 × 40 m) were randomly established in each plantation (Figure. 1). In total, 60 plots were established across four districts. In each sample plot, 30 trees were randomly selected for scoring damage index. The damage was ranked into five levels, where: 0 = trees without damage; 1 = trees with <25% leaves damaged; 2 = trees with 25 to <50% leaves damaged; 3 = trees with 50 to <75% leaves damaged; 4 = trees with more than 75% leaves damaged.

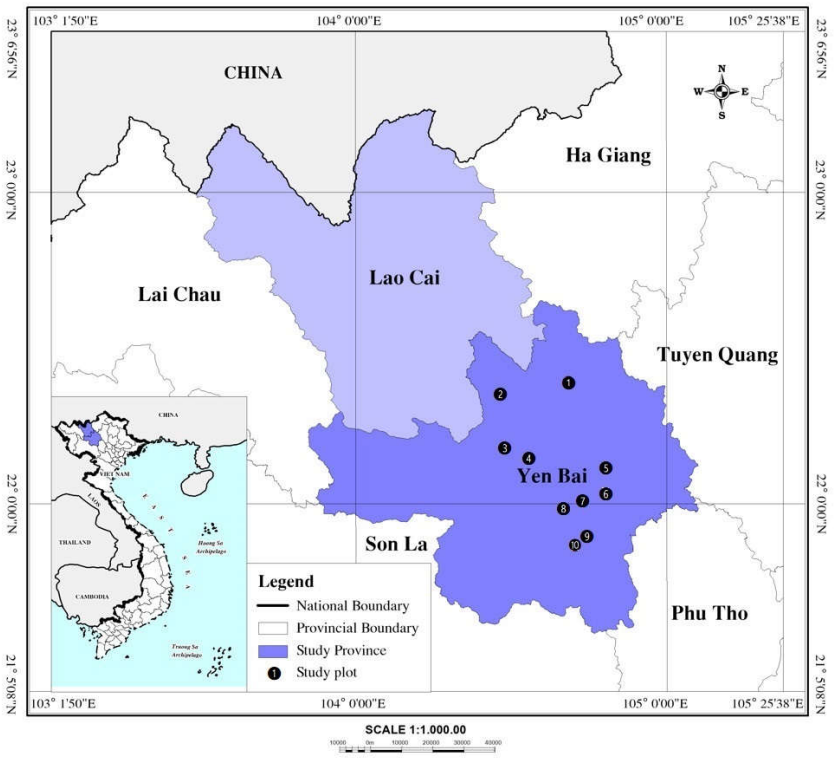

Figure. 1. Geographic location of *Cinnamomum cassia* plantations infested with *Cricula variabilis* in Yen Bai province, Vietnam where study sites were established

2.4. Data analysis

Following the results of damage classification, the damage incidence and damage index were calculated following Nguyen and Dao (2004) [16] and McMaugh (2005) [17], as follows:

$$P\% = (n/N) \times 100 \quad (1)$$

Where: P% = damage incidence; n = the number of trees attacked by *C. variabilis*; N = total number of trees assessed;

$$DI = (\Sigma n_i \times v_i)/N \quad (2)$$

Where: DI = damage index; ni = the number of trees infested at damage index i; vi = the damage

index at level i; N = total number of trees assessed. Based on the average damage index, the level of damage severity was ranked at 5 levels: DI = 0, no damage; 0 < DI ≤ 1, light damage; 1 < DI ≤ 2, moderate damage; 2 < DI ≤ 3, high damage; 3 < DI ≤ 4, very high damage.

3. RESULTS

3.1. Identification

Male and female adults (Figure 4) collected from Yen Bai province had almost the same morphology, colour and size as described for the type collection of *Cricula variabilis* [11] and in later publications [12], [13], [18]. Male genital structures (Figure 4) also matched the description of Naumann and Löffler (2010) [11]. Furthermore, the genitalia match those illustrated by Chen *et al.* (2019) [12].

The molecular work showed that the CO1 sequence of male M001 was 100% identical to that of female M002 and both sequences were 98.4% identical to the CO1 sequence of *Cricula variabllis* with the reference number SASNB201-09 on BOLDSYSTEM (Specimen Record | Public Data Portal | BOLDSYSTEMS).

Morphologically, the species of *Cricula* damaging *Cinnamomum cassia* plantations in Yen Bai province is similar to *Cricula variabilis* Naumann & Loffler in China. However, further detailed molecular analysis with a wider collection of Cricula in northern Vietnam is needed to confirm identity with the taxon in China.

3.2. Morphology

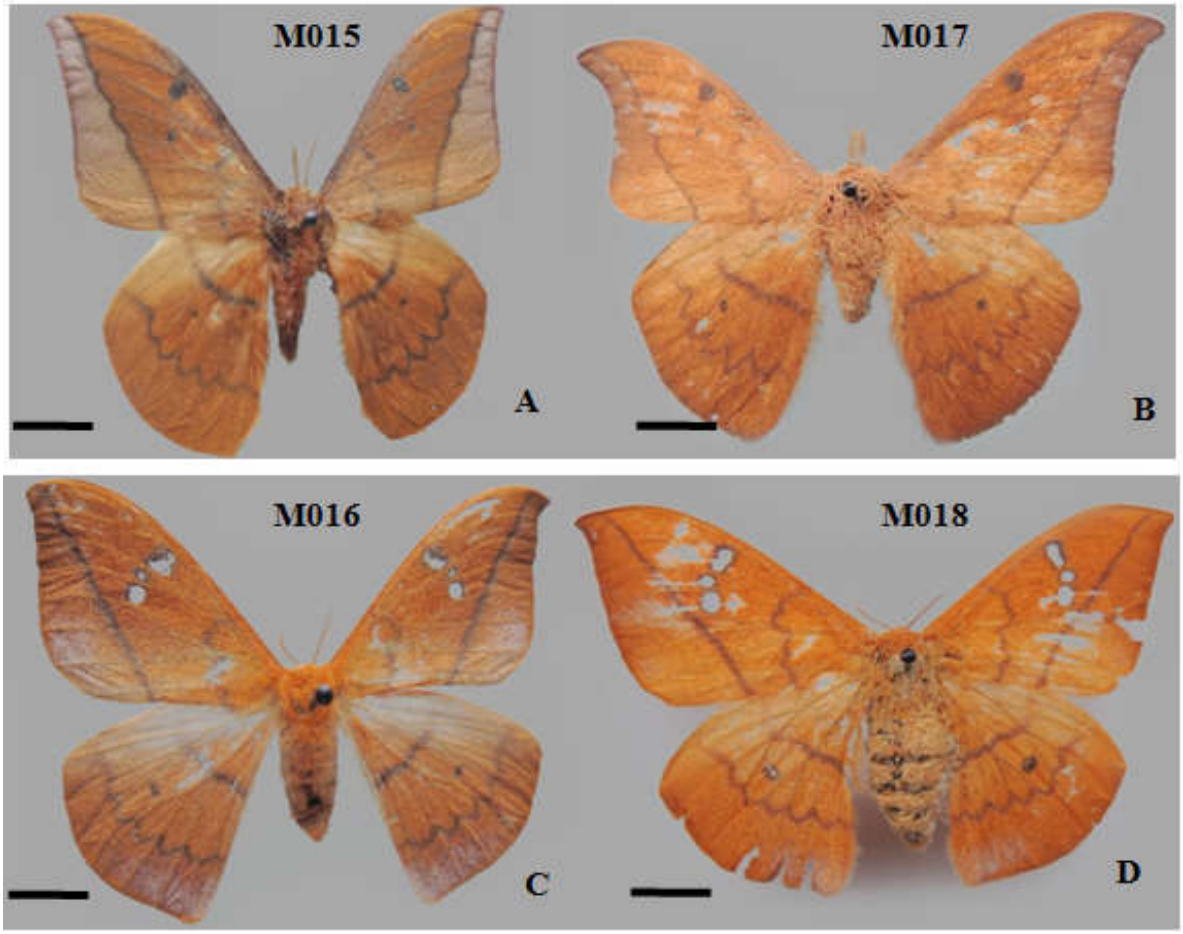

Figure 2. Morphological characteristics of *Cricula variabilis* adults collected from the field in May 2019 (A, C) and July 2019 (B, D). Upper male, lower female, scale bars = 0.5 cm

Figure. 3. *Cricula variabilis* male genitalia. (A) dorsal view with aedeagus removed, (B) aedeagus in lateral view. Scale bars = 0.5 mm

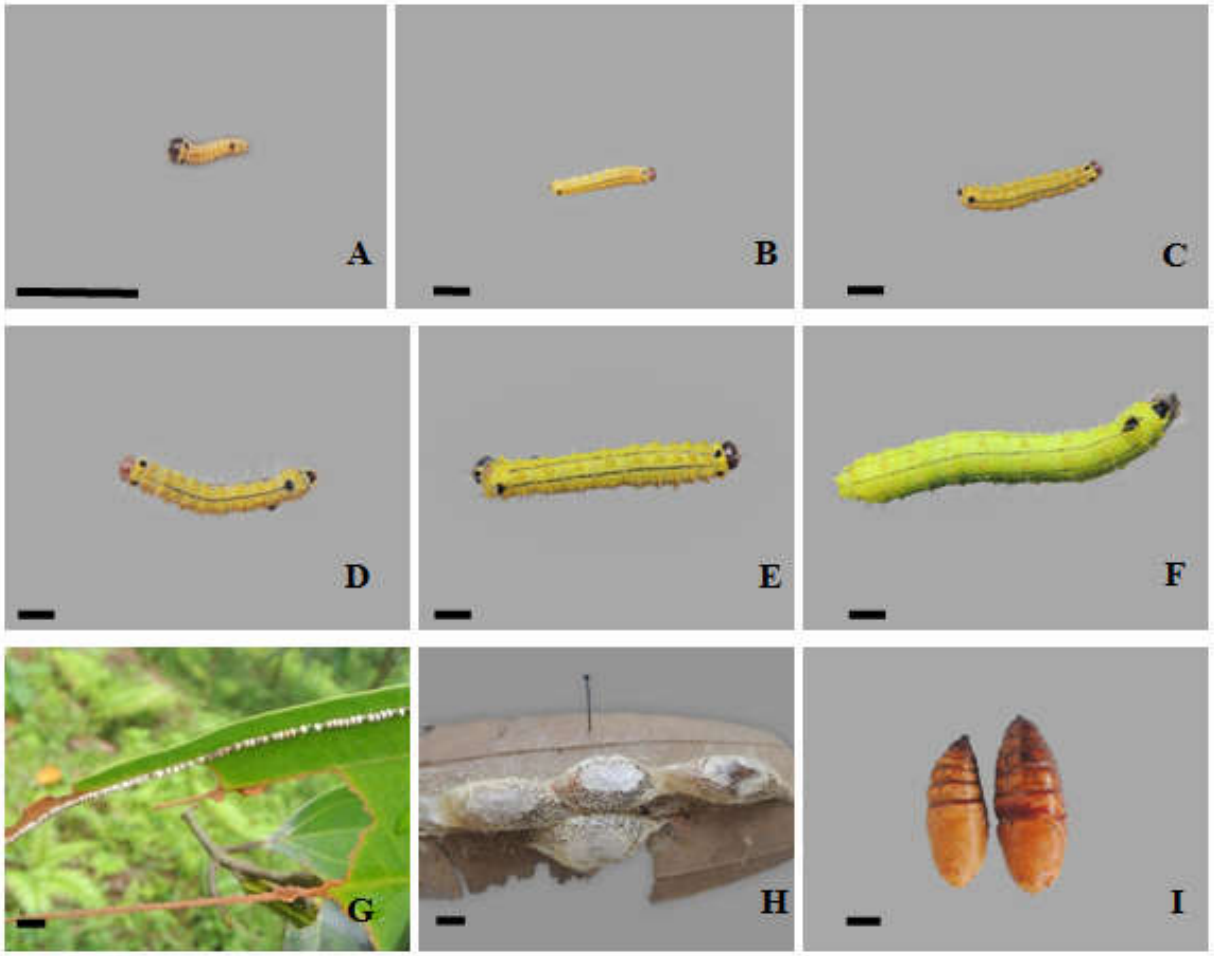

Figure 4. *Cricula variabilis* developmental stages. (A-F) larvae grown in the laboratory: (A) 1st instar, (B) 2nd instar, (C) 3rd instar, (D) 4th instar, (E) 5th instar, (F) 6th instar. (G) Egg and (H) cocoon on *C. cassia* leaf collected from plantation; (I) pupae male (left) and female (right). Scale bars = 0.5 cm

Adults (Figure 2): Body light to dark brown, mostly covered by brown scales, the fore- and hind-wings postmedian are darkened somewhat by grey scales. Wings triangular with acute forewing apex. Postmedian line with slight outward apical curvature. *Male* (Figure. 2A, C): Forewing 28.5-32 mm (mean 30.7±5.7 mm) in length, with up to four fenestrae. Antennae feather-shaped, dark brown, 4.0-8.0 mm in length (mean 5.9±1.1 mm). *Female* (Figure. 2B, D): larger than males. Forewing 31.5-43.0 mm (mean 35.2±7.0 mm) in length, with three fenestrae in the forewing and a single fenestrum in

the hindwing. Antennae filiform, dark brown, 7.0-11.0 mm (mean 8.0±1.1 mm) in length. Male genitalia (Figure. 3): Uncus short with two lateral, almost triangular acute processes, gnathos trapezoid, juxta with two short lateral triangular processes, valva pubescent, phallus with four acute and slender dorsal spines.

Eggs (Figure 4G)*:* oval, ivory white then becoming grey white, 1.5±0.13 mm in length, 1.2±0.2 mm in width. The eggs are laid in one or two parallel rows along the leaf.

Larvae (Figure 4A-F): newly emerged larvae brownish grey, becoming yellowish green then pale green by the sixth instar. The body is covered with numerous white hairs. Mean body length: 1st instar (Figure. 4A) 3.4±1.0 mm; 2nd instar (Figure 4B) 14.2±1.5 mm; 3rd instar (Figure. 4C) 20.5±2.4 mm; 4th instar (Figure. 4D) 29.3±3.1 mm; 5th instar (Figure. 4E) 39.8±3.4 mm; 6th instar (Figure. 4F) 50.2±3.8 mm.

Pupae (Figure 4H, I): light brown, enclosed by yellow cocoons, female 24.2±1.5 mm in length and 12.3±0.9 mm in width, male 19.6±1.2 mm in length and 9.0±0.9 mm in width.

3.3. Life cycle

In the laboratory study, the life cycle took 69.6 days ± 5.2 days, females laid 200-310 eggs during an oviparous period, eggs hatched after 8-11 days, and the larval and pupal periods were 38-48 and 9-15 days, respectively. Adults survived 4-8 days. Newly hatched larvae fed on eggshells before moving away to search for young leaves and start feeding from the edge of the leaf. In the field, larvae often fed in the morning and late afternoon, and moved down to seek shelter to avoid intense sunlight (Figure 5C). Pupae are usually located on the lower leaf surface, branches, or stems. Females mainly emerge during the day and males at night.

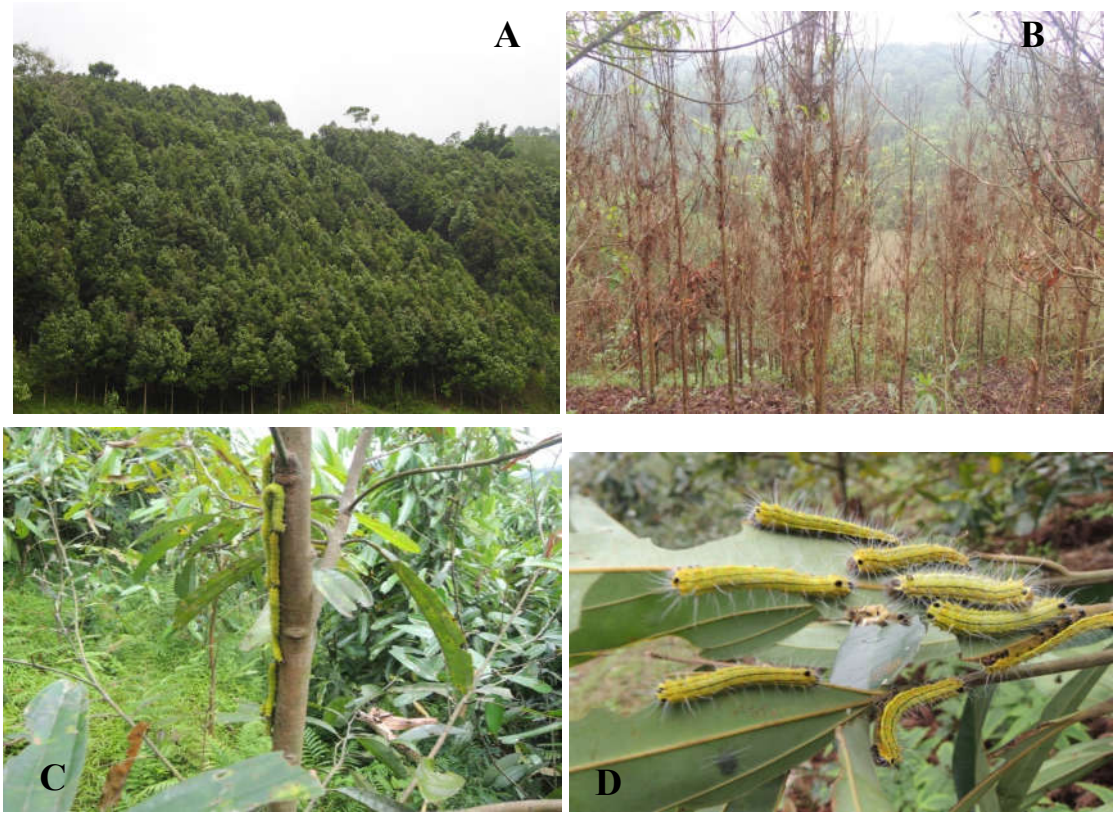

Figure. 5. (A) Healthy *Cinnamomum cassia* plantation, (B) Damaged plantation with most of its foliage consumed by caterpillars, (C) Larva of *Cricula variabilis* moving down the bole to avoid sunlight, (D) fifth instar larvae feeding on leaves

3.4. Damage to *Cinnamomum cassia* plantations

Infestation levels (P%) and damage index (DI) of *C. variabilis* in all ten plantations in Yen Bai province in 2019 was ranged from 47.2 to 71.5% and 1.03 to 2.14, respectively. The most serious damage was in 8-year-old plantations in Tran Yen district where the infestation level (P%) was 71.5±6.5% and average damage index was 2.14±0.21 (Table 1).

Table 1. Plantation details and extent of damage from *Cricula variabilis* in 2019

Location (district)*	Age (year)	Area (ha)	Coordinates	Density (tree/ha)	Elevation (m)	Damage incidence (P%)	Damage index (DI)
Luc Yen[1]	13	6	22°06'66" N 104°76'96" E	1,650	328	47.2±5.10	1.03±0.10
Van Yen[2]	7	6	21°85'16" N 104.61'76" E	3,300	443	66.1±6.2	2.03±0.19
Van Yen[3]	4	7	21°85'14" N 104°60'77" E	6,600	254	53.2±4.6	1.52±0.13
Van Yen[4]	13	5	21°84'25" N 104.60'68" E	1,650	330	51.1±5.0	1.41±0.11
Van Yen[5]	8	5	21°84'30" N 104°61'93" E	3,300	379	59.5±5.2	1.44±0.13
Tran Yen[6]	7	8	21°82'12" N 104°78'64" E	3,300	230	66.2±6.4	1.45±0.16
Tran Yen[7]	8	8	21°73'64" N 104°80'94" E	3,300	165	71.5±6.5	2.14±0.21
Tran Yen[8]	12	4	21°80'87" N 104°80'85" E	1,650	262	54.4±5.4	1.43±0.13
Tran Yen[9]	5	5	21°733'9" N 104°80'72" E	6,600	182	55.3±4.8	1.51±0.14
Van Chan[10]	7	6	21°44'61" N 104°79'88" E	3,300	427	51.6±4.2	1.24±0.12

*Note: * The numbers refer to the locations given in Fig. 1, P% is damage incidence, DI is mean damage index. Values are mean (n = 6) ± SE*

4. DISCUSSION

This is the first report of a *Cricula* species defoliating *Cinnanomum* cassia plantations in Vietnam. Adults have morphological characteristics similar to *Cricula variabilis* Naumann & Loffler collected in central China [11], [12], [13], [18]. The CO1 sequence of the two samples (M001, M002) in this study had 98.4% identity to the CO1 sequence of *Cricula variabilis* (SASNB201-09) on BOLDSYSTEM. The latter specimen was collected by V. Siniaev in Guangxi province (China) in 2008, and deposited in the Research Collection of Stefan Naumann (http://www.boldsystems.org/index.php/Public_RecordView?processid=SASNB201-09). A molecular study, with a number of gene regions across a large number of specimens from different geographical areas, is required to determine the extent to which the taxon in Vietnam is distinct from the type collection.

The *C. variabilis* samples in this study were collected at low (165-465 m) elevations in Yen Bai province, from where it is likely to be spreading to nearby provinces. In China, the larvae of C. variabilis damaged host trees at altitudes from 680 to 840 m above sea level in Guangdong province [12]. There, Phoebe bournei (Lauraceae) is the main host tree, and when all leaves of this host are eaten the larvae move to other tree species such as *Callicarpa kochiana*, *Castanopsis fissa*, *Mucuna pruriens* and *Schefflera octophyyla* [12]. In south China, Zhang *et al.* (2020) [13] reported that members of the Lauraceae and Fagaceae were damaged, including *Cinnamomum burmanni, C. camphora,* and *Castanopsis fissa*. Also, Jin-kun *et al.* (2020) [19] reported that *C. variabilis* damages Machilus chinensis in China.

Studies on the genus *Cricula* have been carried out for many years in Vietnam [11], [20] but this is the first to report of *C. variabilis* in Vietnam. In Vietnam there are 11 species of *Cricula* [11], [20]. Three species (*C. trifenestrata*, *C. jordani* and *C. vietnama*) have been recorded in Tam Dao for a long time [21]. Eight species were added to the record by Nässig *et al.* (2010) [22]. These authors collected *C. falcata* and *C. hoffinanni* in Lam Dong province in the Central Highlands; *C. frederkingi, C. griseorubrescens* and *C. sponai* in Thua Thien - Hue province in Central Vietnam; *C. fansipanensis* and *C. schintlmeisteri* in Lao Cai province in the highlands of Northwest Vietnam; and *C. hoabinhnguyeni* in Bac Kan and Cao Bang provinces in North Vietnam. Most specimens were collected during the cold season from October to December, some even during snow fall. According to surveys conducted by the Forest Protection Research Centre, *C. variabilis* was first seen in a *C. cassia* plantation in Tran Yen district, Yen Bai province in 2016. It is not known whether *C. variabilis* is an alien invasive species or whether there has been opportunistic population buildup of previously undetected natural populations. It is worth noting that the forest protection surveys undertaken by the Forest Protection Research Centre have tended to focus on major pests and small outbreaks could be missed. It appears that the pest is spreading quickly and thus has the potential to threaten *C. cassia* plantations more broadly in Northwest Vietnam. Therefore, there is a need for further studies to determine the geographical extent of *C. variabilis* in Vietnam, to define damage thresholds and to devise integrated management solutions. These are necessary to effectively manage this pest, reduce or prevent its spread, and to safeguard the incomes of rural households.

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ENTOMOPATHOGENIC FUNGI (*Cordyceps* spp.) ISOLATE FROM AMERGING INSECT PESTS ASSOCIATED WITH PLANTATION FORESTS IN VIET NAM

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ABSTRACT

Plantation forests accounted for 4.4 million *hectares* out of 14.7 million *hectares* of total forest area in Vietnam and contribute greatly to the export of timber and wood products as well as the livelihoods of the mountainous people. Several species of insect pests have been observed in *Dendrocalamus barbatus*, *Chukrasia tabularis*, *Acacia* and *Eucalyptus* plantations. However, these pests were found to be parasitized by entomopathogenic fungi. The aims of this study were to examine the pathogenicity and identify the taxonomy of entomopathogens obtained from *Ceracris kiangsu*, *Episparis tortuosalis*, *Batocera lineolata* and *Endoclita* sp. Results showed that seven isolates of the entomopathogenic fungi were found from these four pests. In addition, all identified isolates expressed pathogenicity in *Galleria mellonella* larvae with the parasitism range was from 73 to 100%. Based on ITS, LSU and TEF1-α sequence analysis, the identified entomopathogenic fungi belong to the genus *Cordyceps*. This finding suggests a great potential for application of these *Cordyceps* isolates in insect pest management programs in Vietnam.

Keywords: *Batocera lineolata, Ceracris kiangsu, Endoclita sp., entomopathogenic fungi, Episparis tortuosalis.*

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1. INTRODUCTION

Vietnam has about 4.4 million hectares of planted forests comprised of acacias and eucalypts, which are the exotic species with an estimated area of 2,000,000 and 400,000 ha, respectively [1]. In addition, *Dendrocalamus barbatus* and *Chukrasia tabularis* are native species grown widely with an estimated area of 120,000 and 35,000 hectares [1]. In recent years, forest tree species are frequently attacked by different folivores and stem borers which have been identified as *Ceracris kiangsu* Tsai (Orthoptera: Acrididae) in *D. barbatus* [2], *Episparis tortuosalis* Moore (Lepidoptera: Erebidae) in *C. tabularis* [3], *Batocera lineolata* Chevrolat (Coleoptera: Cerambycidae) in *Eucalyptus* hybrid (*E. urophylla* × *E. grandis*) [4] and *Endoclita* sp. in *Acacia* and *Eucalyptus* plantations (Unpublished data). However, these insect pests were found to be parasitized by the entomopathogenic *Cordyceps* spp., with the rate of 10-15% [2, 5].

Various species of entomopathogenic fungi have been isolated and identified from parasitized insects such as *Beauveria bassiana* from *Cephalcia*

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tannourinensis [6], *B. lii* from *Henosepilachna vigintioctopunctata* [7], and *B. sinensis* from Geometridae (Lepidoptera) [8]. *Cordyceps javanica* and *C. fumosorosea* from *Bemisia tabaci* [9] and *Diaphorina citri* [10]. Several entomopathogens have been used effectively in the biological control of different insect pests [11, 12, 13]. For example, *B. bassiana* and some species of the genus *Cordyceps* were used as microbial insecticides [14, 15]. Because the use of biological agents has been reduced the use of chemical pesticides [12, 13]. Management strategies for plantation diseases and insect pests in Vietnam is also prioritized towards the approach of integrated pest management (IPM), especially using biological agents [16]. This paper aimed at pathogenicity assessment and identification of *Cordyceps* spp. isolated from *C. kiangsu*, *E. tortuosalis*, *B. lineolata* and *Endoclita* sp.

2. MATERIALS AND METHODS

2.1. Sampling, isolation, and culture

In June 2021, seven insect samples of *Batocera lineolata* (1 individual), *Endoclita* sp. (2 individuals), *Episparis tortuosalis* (2 individuals) and *Ceracris kiangsu* (2 individuals) parasitized at the larval, pupal and nymph stages were collected in *Acacia mangium*, *Eucalyptus* hybrid, *Dendrocalamus barbatus*, *Chukrasia tabularis* plantations in Hoa Binh, Phu Tho, Thanh Hoa and Nghe An provinces (Table 1).

Table 1. Entomopathogens obtained from developmental stages of insect pests at different sites

Sample	Insect pest	Stage	Plantation	Location
B2	*Endoclita* sp.	Larva	*Acacia mangium*	Tan Lac, Hoa Binh
B3	*Endoclita* sp.	Larva	*Eucalyptus* hybrid	Yen Lap, Phu Tho
B4	*Batocera lineolata*	Pupa	*Eucalyptus* hybrid	Yen Lap, Phu Tho
B5	*Ceracris kiangsu*	Nymph	*Dendrocalamus barbatus*	Muong Lat, Thanh Hoa
B6	*Ceracris kiangsu*	Nymph	*Dendrocalamus barbatus*	Muong Lat, Thanh Hoa
B7	*Episparis tortuosalis*	Larva	*Chukrasia tabularis*	Nghia Dan, Nghe An
B8	*Episparis tortuosalis*	Larva	*Chukrasia tabularis*	Nghia Dan, Nghe An

Conidia from the surface of each parasitized sample were transferred to Potato Dextrose Agar (PDA) medium in Petri dishes with diameter of 9 cm, and incubated at 25-26°C in a laboratory. After 5-6 days, when cultures had grown 0.5-1.0 cm in diameter, hyphal tips were subcultured onto new dishes and incubated at 25-26°C. All isolates were stored at the Forest Protection Research Centre, Vietnamese Academy of Forest Sciences.

2.2. Pathogenicity tests on larval *Galleria mellonella*

Initial test was carried out to examine the efficacy of seven entomopathogenic fungal isolates on the 4th larval instars of *Galleria mellonella*

fowllowing the method of Hussein *et al.* (2012) [17]. Conidia of the 14 day-old fungal cultures grown on PDA at 25°C were harvested by scraping the sporulating colonies and suspending in distilled water. The conidial suspension was filtered through two layers of cheesecloth. The spore solution was diluted at a density of about 5×10^6 CFU/ml + 1% Tween 80. *Beauveria bassiana* (Muskardin, dosage of 3.75 g/l) + 1% Tween 80 was applied in the test as a positive control. Steriled water + 1% Tween 80 were also included as negative control. The larvae were submersed for 5s in each treatment. In other test, the treatments were sprayed on the larvae at a dosage of 3 ml/treatment.

In each test, four replicates of each treatment were carried out using fifteen larvae per replicate. After treatment, the larvae were placed in a sterilized moist chamber consisting of a plastic box (9 cm in diameter, 3 cm in depth) with wet filter paper, and incubated at 25-26°C. The number of parasitized larvae were counted after two, three, four days of the direct inoculation and three, six, nine days of the spraying.

2.3. Sequencing and phylogenetic analysis

The primers ITS1/ITS4, 5.8SR/LR7 and EF983F/EF2212R were used to identify the entomopathogenic fungal isolates through ITS, LSU and TEF1-α gene amplification. Amplifications were carried out in 49-µl-volume reactions containing 20 µl Master Mix (Eppendorf, Germany), 1 µl of each forward and reverse primer, 1 µl of DNA template and 27 µl sterilized water. The PCRs were performed with a C1000 Touch™ thermal cycler (Bio-Rad, USA). The PCR cycling parameters were as follows: initial denaturation for 3 minutes at 94°C, followed by 30 cycles at 94°C for 30 seconds, 52°C for 30 seconds and 72°C for 1 minute. The amplification was completed at 72°C for 10 minutes and then the PCR product was stored at 10°C. The PCR amplicons were sequenced at 1st BASE (Malaysia). The DNA sequences were compared to the GenBank database via the nucleotide-nucleotide BLAST search interface located at the National Center for Biotechnology Information, Bethesda, USA. Relevant sequences were transferred and then processed using BioEdit software [18].

Phylogenetic analyses were perfomed using the ITS, LSU and TEF1-α sequences. The evolutionary history was inferred by using the Maximum Likelihood method based on the Kimura 2-parameter model [19]. Consensus trees with the highest log likelihoods (1158.71) were created. Evolutionary analyses were conducted in MEGA7 [20]. *Ophiocordyceps sinensis* was used as the outgroup taxon to root the tree.

2.4. Data analysis

The parasitized rate (*P%*) was calculated according to the following equation:

$$P\% = (n/N) \times 100$$

Where: *n* is the number of parasitized insects; *N* is total number of insects assessed.

Data were analysed using GenStat Release 12.1 software package. Analysis of variance was used to test for significant effect of blocks and treatments, followed by Duncan's Multiple Range Test for comparisons of means of different treatments.

3. RESULTS AND DISCUSSION

3.1. Isolation

Parasitized *C. kiangsu* nymphs and *E. tortuosalis* larvae were commonly observed on ground arround the tree crown projection of *D. barbatus* or *C. tabularis,* where humidity is relatively high (Figure 1c). Parasitized *Batocera lineolata* pupa (Figure 1a) and *Endoclita* sp. larvae (Figure 1b) were found in their breeding galleries inside the stem of *Acacia* and *Eucalyptus* trees. Their bodies were dry and covered with numerous white conidia.

Seven entomopathogenic fungal isolates were found in the parasitized insect samples (Table 1) including *Endoclita* sp. (2 isolates), *B. lineolata* (1 isolate), *C. kiangsu* (2 isolates) and *E. tortuosalis* (2 isolates).

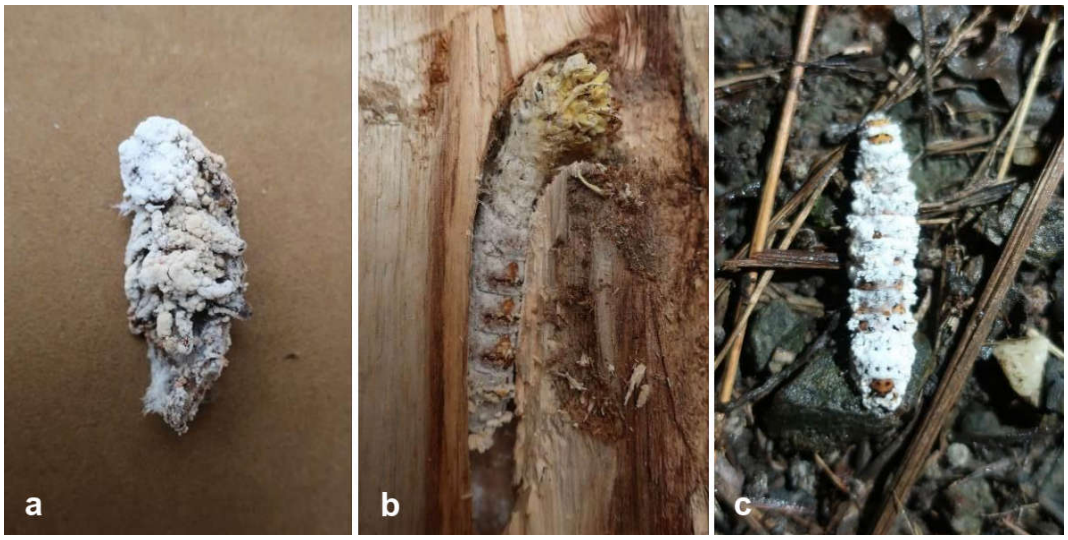

Figure 1. Parasitized insects in forests: a. Pupa of *Batocera lineolata*, b. Larva of *Endoclita* sp., c. Larva of *Episparis tortuosalis*

3.2. Pathogenicity on larval *Galleria mellonella*

There was no significant difference between blocks ($P > 0.05$) in the direct inoculation test and the spraying test (data was not shown). All tested entomopathogenic isolates had pathogenicity on *G. mellonella* larvae (Table 2). The percentage of larvae parasitized by seven isolates (B2, B3, B4, B5, B6, B7 and B8) in the first test method of direct inoculation after two and three days were 51.7-65.0% and 63.3-76.7%, respectively. After four days, the percentage of parasitized larvae reached 85-100%. Three isolates (B6, B7 and B8) infected and covered the whole larvae, similar to the Muskadin (positive control).

Table 2. Percentage of *Galleria mellonella* larvae parasitized in two infection methods

Treatment	Days after direct inoculation (%)			Days after spraying (%)		
	2	3	4	3	6	9
B2	51.7 ± 1.6^{b}	63.3 ± 1.7^{b}	85.0 ± 2.6^{b}	26.7 ± 0.6^{b}	53.3 ± 2.2^{b}	73.3 ± 2.7^{b}
B3	60.0 ± 2.1^{cde}	71.7 ± 2.4^{cd}	93.3 ± 3.1^{c}	30.0 ± 0.9^{b}	63.3 ± 1.8^{cd}	76.7 ± 2.9^{bc}
B4	55.0 ± 1.3^{bc}	65.0 ± 1.7^{bc}	86.7 ± 2.2^{b}	25.0 ± 1.2^{b}	55.0 ± 2.4^{bc}	76.7 ± 3.0^{bc}
B5	60.0 ± 3.2^{cde}	70.0 ± 2.9^{bcd}	93.3 ± 4.1^{c}	26.7 ± 1.7^{b}	61.7 ± 3.3^{bcd}	78.3 ± 3.6^{bc}
B6	61.7 ± 3.5^{cde}	75.0 ± 3.4^{d}	100^{d}	38.3 ± 1.9^{bc}	65.0 ± 2.7^{d}	80.0 ± 2.8^{bc}
B7	65.0 ± 2.8^{e}	76.7 ± 3.8^{d}	100^{d}	50.0 ± 1.6^{c}	68.3 ± 2.4^{d}	81.7 ± 3.5^{c}
B8	63.3 ± 1.9^{de}	75.0 ± 4.5^{d}	100^{d}	41.7 ± 1.3^{bc}	66.7 ± 2.6^{d}	80.0 ± 3.0^{bc}

Treatment	Days after direct inoculation (%)			Days after spraying (%)		
	2	3	4	3	6	9
Muskardin	56.7±1.6^{bcd}	73.3±4.2^{d}	100^{d}	26.7±1.5^{b}	60.0±4.2^{bcd}	76.7±4.3^{bc}
Water	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}
P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Notes. Values identified by the same letters in a column are not significantly different among treatments at the 0.05 level according to Duncan's multiple range tests

The percentage of parasitized larvae by seven isolates (B2, B3, B4, B5, B6, B7 and B8) after spraying three, six and nine days were 25-50%, 53.3-68.3% and 73.3-81.7%, respectively. The parasitic effect of the entomopathogenic fungi was slower in the spraying test than the direct inoculation test. After nine days, the percentage of parasitized larvae by the spraying (Figure 2c) was higher in the isolate B7 than the Muskadin (Figure 2d).

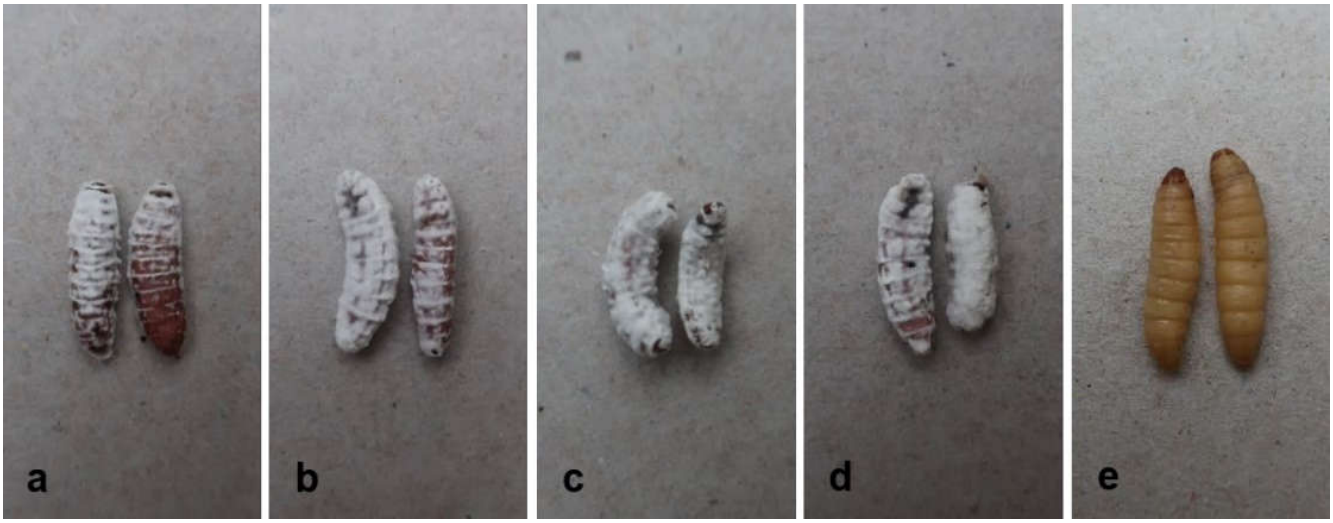

Figure 2. Parasitized *Galleria mellonella* larvae after nine inoculation days: a-d. different isolates; e. controls; a. isolate B2; b. isolate B3; c. isolate B7; d. Muskardin; e. water

3.3. Identification

The ITS, LSU, and TEF1-α gene sequences of seven isolates in this study were compared with reference sequences obtaned from the National Center for Biotechnology (NCBI) GenBank for *Cordyceps bifusispora*, *C. blackwelliae*, *C. chiangdaoensis*, *C. cicadae*, *C. coleopterora*, *C. farinose*, *C. fumosorosea*, *C. ghanensis*, *C. jakajanicola*, *C. lepidopterorum*, *C. militaris*, *C. morakotii*, *C. ninchukispora*, *C. pruinosa*, *C. rosea* and *C. tenuipes* (Table 3). Bootstrap values were equal to or greater than 50% derived from 1000 iterations.

The isolate B2 and B3 did not match to the data available on the NCBI. Five isolates including B4, B5, B6, B7, and B8 was only 98% identical to the *Cordyceps jakajanicola* (Figure 3). In addition, the morphological and sporal characteristics of these isolates are not similar to those of *Coryceps* species that have been recorded previously. These isolates are probably new species and further studies are required to clarify this point.

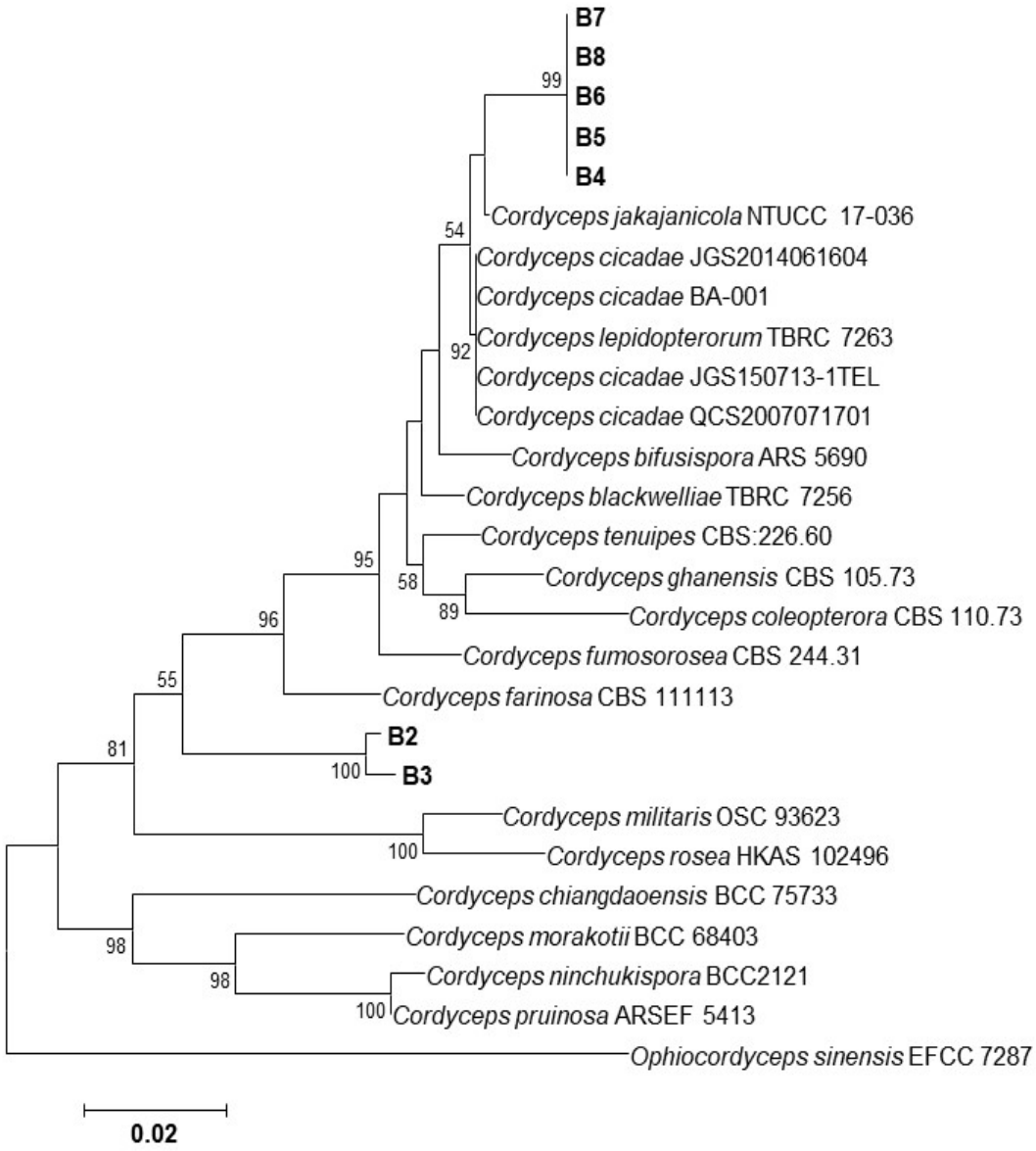

Figure 3. Consensus tree of the concatenated sequences of ITS, LSU and TEF1-α from different species of the genus *Cordyceps*. The Maximum Likelihood method was used to construct the tree. The bar represents an expected sequence variation of 2.0%. *Ophiocordyceps sinensis* was used as the outgroup taxa to root the tree

Table 3. GenBank accession numbers of *Cordyceps* isolates in this study

Species	Isolate	ITS	LSU	TEF1-α
Cordyceps sp.	B2	ON876733	ON876740	ON815106
Cordyceps sp.	B3	ON876734	ON876741	ON815107
Cordyceps sp.	B4	ON876735	ON876742	ON815108

Species	Isolate	ITS	LSU	TEF1-α
Cordyceps sp.	B5	ON876736	ON876743	ON815109
Cordyceps sp.	B6	ON876737	ON876744	ON815110
Cordyceps sp.	B7	ON876738	ON876745	ON815111
Cordyceps sp.	B8	ON876739	ON876746	ON815112
C. bifusispora	ARS 5690	AY245627	EF468806	EF468746
C. blackwelliae	TBRC 7256	NR_164416	MF140702	MF140822
C. chiangdaoensis	BCC 75733	KT261397	MZ573231	KT261407
C. cicadae	JGS2014061604	KX017276	MT239107	MK770632
C. cicadae	BA-001	AEIW01001963	AEIW01001963	AEIW01000182
C. cicadae	JGS150713-1TEL	MT192488	MT239107	MT637809
C. cicadae	QCS2007071701	KX017277	MK761212	MK770631
C. coleopterora	CBS 110.73	AY624177	JF415988	JQ425689
C. farinose	CBS 111113	AY624181	MF416554	MF416499
C. fumosorosea	CBS 244.31	AY624182	MF416557	MF416503
C. ghanensis	CBS 105.73	NR_111171	MH872340	MN338483
C. jakajanicola	NTUCC 17-036	MT966043	MT974255	MW025836
C. lepidopterorum	TBRC 7263	MF140765	MF140699	MF140819
C. militaris	OSC 93623	JN049825	AY184966	DQ522332
C. morakotii	BCC 68403	KT261392	MZ573234	KT261402
C. ninchukispora	BCC 2121	FJ765277	FJ765245	FJ765261

Species	Isolate	ITS	LSU	TEF1-α
C. pruinosa	ARSEF 5413	JN049826	AY184968	DQ522351
C. rosea	HKAS 102496	MT012344	MT012351	MT025050
C. tenuipes	CBS:226.60	MH857959	LUFF01000002	LUFF0100007

3.4. Discussion

This study is the first report of the *Cordyceps* parasitic entomopathogens found in *Batocera lineolata*, *Ceracris kiangsu*, *Endoclita* sp. and *Episparis tortuosalis* in Vietnam. Many parasitic fungi species have been found in insect pests such as *B. bassiana* from *Cephalcia tannourinensis* [6], *Cordyceps javanica* and *C. fumosorosea* from *Bemisia tabaci* [9], *Diaphorina citri* [10], and *B. lineolata* [21]. *C. wuyishanensis* and *C. maolanoides* from cicada and dung beetles [22]. *Ophiocordyceps macroacicularis* and *O. ramosissimum* from *E. davidi* [23].

Different species of *Cordyceps* spp. have not only high medicinal roles [14, 23], but also effective biopesticides [14, 15, 21]. Some entomopathogenic fungi are effective to insect pests [11, 12, 13]. In Switzerland, *B. brongniartii* infection rates of *Melolontha melolontha* was 59-86% [24], and larvae survived in the soil after 16 months of the fungal application [25]. *B. bassiana* and *B. brongniartii* have been shown to be safe entomopathogenic fungi [13]. *B. bassiana* strains 147 were used as an insecticide [11]. *B. brongniartii* and *B. bassiana* were used for the control of *Brahmina coriacea* with the dead larvae rate ranged from 40 to 83% [26]. *Metarhizium anisopliae* and *Isaria fumosorosea* [15] and *C. fumosorosea* [27] were used for the management of *D. citri* in the field. *C. cateniannulata* were used to cotrol of *Spodoptera frugiperdain* in China [28].

Seven *Cordyceps* isolates observed from the parasitized samples of *Endoclita* sp., *B. lineolata*, *C. kiangsu*, and *E. tortuosalis* in this study had high effectiveness when being inoculated into *Galleria mellonella* larvae. The percentage of parasitized *G. mellonella* larvae in the direct inoculation and spraying experiment were 73.3-81.7%, and 85-100%, respectively. *C. bassiana* had a high effective against the nymphs and adults of *D. citri* in the field [15]. *Cordyceps fumosorosea* was used for management of *Bemisia tabaci* [29, 9]. *C. javanica* was used for management of *Diaphorina citri* in the field with effectiveness of 60-90% [30, 10], and *B. lineolata* in China [21].

The *Cordyceps* isolates identified in this study would be potential for management plans of insect pests in Vietnam. Although these isolates were sequenced by the ITS, LSU, and TEF1-α genes, but other primers are recommended for specific identification of the enthopathogenic fungal species.

4. CONCLUSION

Seven entomopathogenic fungal isolates were isolated from *Endoclita* sp. (2 isolates), *B. lineolata* (1 isolate), *C. kiangsu* (2 isolates) and *E. tortuosalis* (2 isolates). These seven isolates were pathogenic to *G. mellonella* larvae with the percentage of parasitism ranged from 73.3 to 100% in both spraying and direct inoculation methods. Based on sequence analysis of ITS, LSU and TEF1-α, all seven entomopathogenic fungi were identified to be *Cordyceps* spp.

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APPLICATION OF ANALYTIC HIERARCHY PROCESS (AHP) IN DEVELOPING ECO-SUSTAINABILITY CRITERIA FOR THE COFFEE FARMING ASSESSMENT: A CASE STUDY IN DAK HA DISTRICT, KON TUM PROVINCE

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ABSTRACT

Dak Ha is one of the nine districts in Kon Tum province, covering an area of more than 84 thousand hectares, and has the largest coffee area in Kon Tum province. The study aims to develop a set of Eco-Sustainability criteria for the coffee tree model in Kon Tum province. The study employed a combination of methods for data collection and analysis, including interviews with 575 farmers and 15 experts, analytic hierarchy process (AHP) method, land suitability assessment method, multi-criteria analysis (MCA), and synthetic assessment of DPSIR. The findings identified 15 factors affecting the Eco-Sustainability of coffee cultivation in specialized areas with 6 factors having the most influence in the order of cost, profit, capital efficiency, water quantity and quality, soil quantity and quality, and productivity. On that basis, the study proposed programs and solutions to apply Eco-Sustainability criteria to contribute to the development of sustainable coffee production for Kon Tum province.

Keywords: *sustainable ecology, criterion assessment, coffee farming, applicable solution, Kon Tum province.*

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1. INTRODUCTION

The Central Highlands is one of the key economic regions of the country with significant potential for economic development in agriculture and forestry. Kon Tum is a province in the Central Highlands, with a natural land area of 967,729.83 ha [1]. According to data from the Department of Natural Resources and Environment of Kon Tum province (2020), the agricultural land area accounts for 902,391.01 ha (93.25% of the total natural area); while the area for planting perennial crops is 154,362.74 ha (15.74% of the total area) and the area for growing coffee is 25,206 ha [2]. Following the implementation of the province's crop restructuring project, the area of coffee in 2003 increased from 12,833 ha in 2015 to 15,265 ha in 2020 and by the end of 2020 reached over 25,000 ha [3, 4]. However, the rapid expansion of coffee production, which ignores coffee quality, causes many problems in terms of economic efficiency and the ecological environment, threatening the sustainability of coffee production. Currently, there are no clear criteria in Vietnam in general, and in Kon Tum province in particular, to evaluate the Eco-Sustainability of the coffee farming model, resulting in a lack of clarity in reflecting on the current state and making recommendations for

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ensuring the long-term development of coffee trees in terms of economy, society, and the environment. Therefore, it is very important to evaluate the factors affecting Eco-Sustanability in coffee cultivation to build a set of Eco-Sustainability criteria applicable to coffee cultivation. Proposing programs and solutions that apply Eco-Sustanability criteria is required to contribute to the development of Kon Tum province's sustainable coffee production.

2. RESEARCH METHODS

2.1. Research subject

Research location: The study was conducted in Dak Ha district, Kon Tum province.

Research subject: coffee farming households in 11 communes, towns, Dak Ha district, Kon Tum province.

Research date: These estimates are based on data collected over a 10-year period of time (2010-2020).

2.2. Research methods

- Survey method: survey, interview coffee farming households with the number of 575 farmers and 15 experts, details of this method have been presented in the previous article of the author group [5].

- Analytical and evaluation method: to assess the suitability of land for coffee farming in the Dak Ha district [6].

- Synthetic assessment method of DPSIR (Driving Force - Pressure - State - Impact - Responses) [7]: includes the following framework system: Dynamics - Pressure - Status - Impact - Response. The model describes the relationship between motivation - D (increasing socio-economic demands in agricultural production areas), pressure - P (excessive development pressure, waste sources that pollute and degrade the environment), current status - S (environmental quality), impact - I (problems related to changes in the ecological environment affecting people and nature, economic and social), reaction - R (solutions to protect the ecological environment).

- The Multi - Criteria Analysis (MCA) method combines the expert method for determining the set of weights for the criteria with the expert method for quantifying the criteria.

The MCA method has eight steps. However, experts use only steps 1 - 4 and 7 to analyze the decision support criteria for uncomplicated decisions. Matrixes are used to perform multi-criteria analysis.

(+) Determine the decision content. What is the purpose of the MCA method, who is the decision maker, and who is involved in the process?

Establish a hierarchical structure of Eco-Sustainability factors in coffee farming with two levels: level 1 includes three criteria: economic, social, and ecological environment; level 2 includes fifteen component criteria: cost; profit; capital efficiency; productivity; area; financial support; cultivation practices; technical assistance; jobs; the suitability of capital capacity; quantity and quality of water; soil quality; residues of fertilizers and pesticides; reducing biodiversity; increased disease.

The opinions of 15 experts are gathered based on the criteria, and the comparison value of the

pairs of factors at level 1 and level 2 is analyzed, the comparison matrix is established, and the weights of the factors at each level are established.

(+) Determine alternative options.

After determining the partial weights of factors of level 1 and level 2, determine the global weight of the criteria.

(+) Define goals and criteria that reflect the value associated with the outcome of each alternative.

Determining the degree of influence of good factors on Eco-Sustainability in coffee cultivation in specialized areas.

(+) Describe the expected performance of each alternative against the criteria. If combined with steps 5 and 6, describe the effect with a score.

From there, develop a remedial plan for the affected factors.

(+) Assign weights to each criterion to reflect their relative importance to the decision.

(+) Combine weights and scores for each alternative to obtain an overall value.

(+) Check the results.

Based on the criteria that were measured and analyzed hierarchically, a reality check was conducted with the results of survey questionnaires on the farming situation of the people, the land was assessed, and samples were taken to analyze the indicators of soil and water sample components.

Building a map of natural soil adaptation for coffee trees in Dak Ha district, Kon Tum province [6].

(+) Conduct a sensitivity analysis of the results to changes in weights.

Analytical Hierarchy Process (AHP) [8]: functional hierarchical analysis was used to solve the problem in this study. The AHP method measures consistency using the consistency ratio (CR); if the value of the consistency ratio is greater than 10%, the judgment is random and should be repeated.

3. RESEARCH RESULTS

3.1. The set of Eco-Sustainability criteria for the coffee farming model suitable to the natural, economic, and social conditions of Kon Tum province

3.1.1. Factors affecting Eco-Sustainability in coffee cultivation

The results of the survey of 575 farmers in the Dak Ha show that the current farming situation is affected by 3 main factors: socio-economic and ecological environment. According to the research results of Joe Walker (2011), these three criteria can also be used to measure and evaluate the sustainability of the farming area, thereby, providing information to decide on agricultural farming planning [9].

Research results on the current status of coffee cultivation, types of coffee farming models, and economic efficiency of the models have been presented in the previous study of the authors [5].

3.1.2. Set of criteria for assessing Eco-Sustainability for coffee farming models

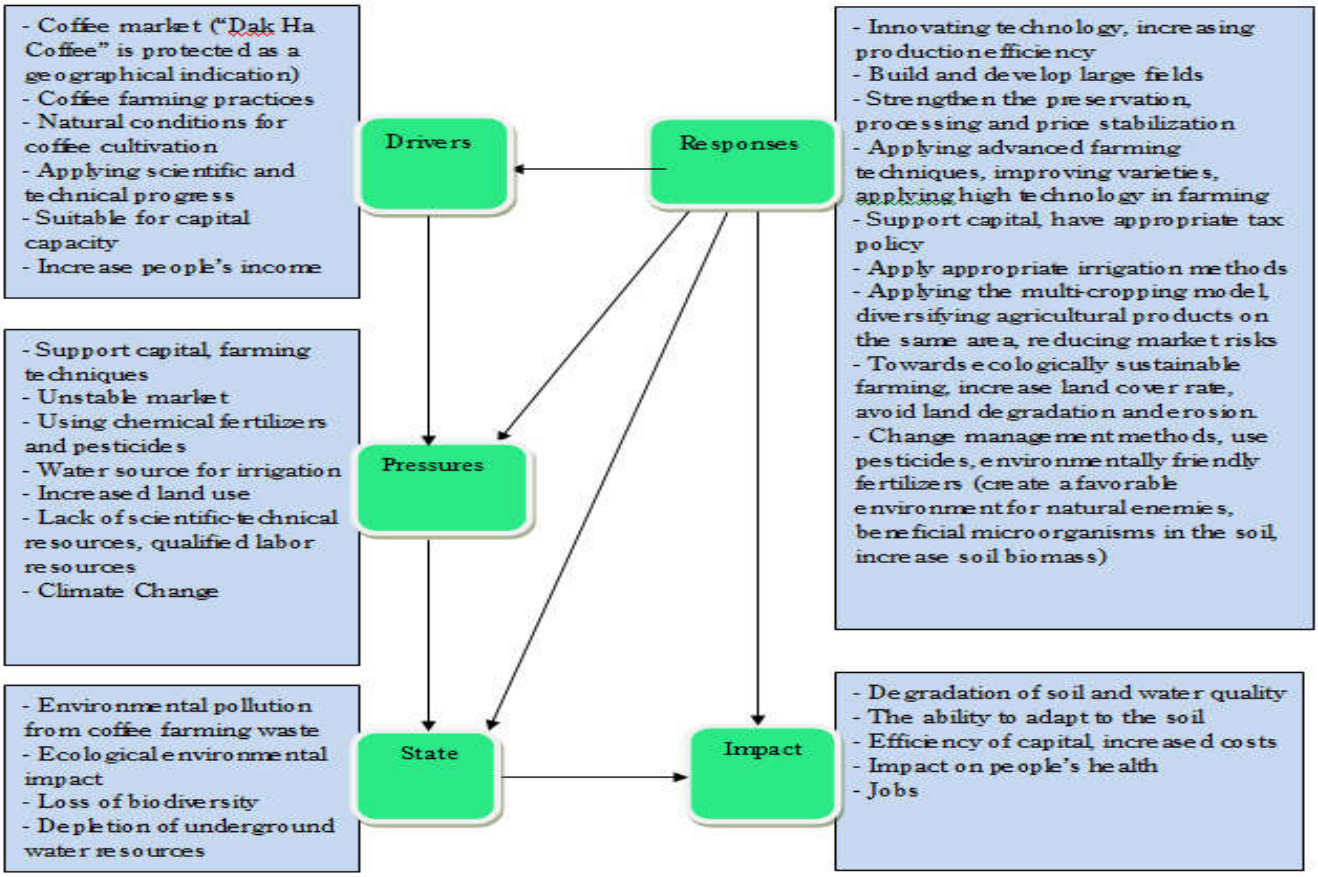

Figure 1. DPSIR diagram in coffee farming in Dak Ha district, Kon Tum province

Based on the DPSIR model analysis and the FAO's sustainable land use index framework, it was determined that 15 factors, divided into two functional levels, to analyze and assess the Eco-Sustanability of the coffee-growing area of Dak Ha, Kon Tum province, through the actual investigation of coffee cultivation in the specialized farming area of Dak Ha, Kon Tum province.

Building a comparison matrix with three factors at level 1 to synthesize factors: economic (KT), social (XH), and ecological environment (MST). Thereby, getting the expert opinion, and calculating the weight of the factors.

Table 1. The hierarchical structure of Eco-Sustainability factors in coffee farming

Level 1	Level 2	Sign	DPSIR	Sustainable land use
Economy (KT)	1. Cost	CP	I	Long term
	2. Profit	LN	I	Long term
	3. Capital efficiency	HQĐV	D	Production efficiency
	4. Productivity	NS	I	Long term
	5. Area	DT	I	Long term
Society (XH)	6. Financial support	HTC	D	Social acceptance
	7. Cultivation practices	TQCT	D	Social acceptance
	8. Technical assistance	HKT	P	Social acceptance
	9. Settlement of jobs	GQVL	I	Social acceptance
	10. The suitability of capital	KNV	P	Social acceptance
Eco-environment	11. Water quantity and quality	WATER	S	Safety
	12. Soil quality	SOIL	S	Safety

(MST)	13. Residues of fertilizers and pesticides	DL	S	Safety
	14. Reducing biodiversity	GDSH	S	Protection
	15. Increasing disease	GTDB	P	Protection

Table 2. Pairwise comparison of level 1 factors of experts

Comparision		Order of expert evaluation results									Aij
i	j	1	2	3	4	5	6	7	8	9	
KT	XH	3	2	4	3	5	6	8	7	8	37/8
	MST	3	2	5	2	3	7	6	5	3	911/250
XH	MST	1/2	1/2	1/2	1/2	1/3	1/2	1/2	1/2	1/5	54/125
CR(%)		4.6	4.6	8.2	0.8	3.3	7.1	1.6	1.2	3.8	3.9

From the results of consulting 15 experts, after synthesizing and analyzing, 9 opinions were selected to synthesize with the CR<10% consistency ratio, so the pairwise comparison matrix is consistent weight set.

Table 3. Comparison matrix and weighting factors of level 1

Criteria	KT	XH	MST	Weight (W)
KT	1	37/8	911/250	0.59
XH	8/37	1	54/125	0.11
MST	250/911	125/54	1	0.20

The results of the general comparison of experts in table 2, the weighting of the factors by the method of eigenvectors, and the determination of the weight vectors (Table 3) are as follows:

$[W_{KT}; W_{XH}; W_{MST}]=[0.59; 0.11; 0.20]$

Level 1 standards for the economy, society, and the natural environment are concretized into level 2 standards for evaluation.

Regarding the economic standard group: Create a matrix to compare five evaluation factors: Cost (CP); Profit (LN); Capital efficiency (HQDV); Productivity (NS); and Area (DT) from experts, select results with CR<10% consistency. The following results:

Table 4. Comparative value of level 2 factor pairs of experts in the economic group

Comparision		Order of expert evaluation results									Aij
i	j	1	2	3	4	5	6	7	8	9	
CP	LN	3	3	2	3	3	3	2	4	2	1353/500
	HQDV	3	4	5	4	3	5	4	5	3	783/200
	NS	4	3	3	3	3	4	5	8	3	151/40
	DT	2	3	5	4	5	5	5	5	4	203/50
LN	HQDV	2	3	4	3	2	4	3	2	3	1397/500
	NS	2	4	3	2	3	3	6	5	5	137/40
	DT	3	4	4	3	4	4	3	3	4	88/25
HQDV	NS	3	2	3	2	3	2	4	4	4	721/250
	DT	3	3	4	3	4	4	3	3	5	699/200
NS	DT	2	2	2	3	3	3	2	2	1	53/25
CR(%)		9.4	8.5	9.3	7.4	6.5	8.9	7.7	8.0	8.2	8.2

Table 5. Comparison matrix and weighting of level 2 factors in the economic group

Criteria	CP	LN	HQDV	NS	DT	Weight (W)
CP	1.00	2.71	3.92	3.78	4.06	0.43
LN	0.37	1.00	2.79	3.43	3.52	0.25
HQDV	0.26	0.36	1.00	2.88	3.50	0.16
NS	0.26	0.29	0.35	1.00	2.12	0.09
DT	0.25	0.28	0.29	0.47	1.00	0.06

Based on the results of the general comparison of experts in Table 4, the weights of the factors were calculated using the separate vector method, and the weight vectors were determined (Table 5) as follows:

[W_{CP}; W_{LN}; $W_{HQĐV}$; W_{NS}; W_{DT}]=[0.43; 0.25; 0.16; 0.09; 0.06]

Regarding the social standard group: Similar to the economic standard group, the social standard group established a matrix comparing 5 evaluation factors: Financial support (HTC); cultivation practices (TQCT); technical support (HKT); Employment settlement (GQVL); matching capital capacity (KNV) from experts, choosing results with CR<10% consistency ratio. The following results:

Table 6. Compared value of level 2 factor pair of experts in the social group

Comparision		Order of expert evaluation results									Aij
i	j	1	2	3	4	5	6	7	8	9	
HTC	TQCT	3	5	3	2	2	3	5	3	5	13/4
	HTKT	2	4	4	4	5	3	3	3	3	3341/1000
	GQVL	5	7	3	3	4	4	4	4	3	991/250
	KNV	4	4	4	4	5	5	5	3	5	2139/500
TQCT	HTKT	1/3	1/3	1/3	2	1/2	1/2	1/3	1/2	1/3	233/500
	GQVL	3	3	2	3	3	5	3	3	2	1451/500
	KNV	2	2	3	3	3	3	2	4	5	358/125
HTKT	GQVL	3	2	2	2	4	3	3	4	2	2671/1000
	KNV	3	3	3	3	3	3	5	3	3	127/40
GQVL	KNV	2	2	3	2	2	2	2	2	2	523/250
CR(%)		5.1	8.8	9.0	3.8	8.7	7.3	6.7	8.0	9.0	7.4

Table 7. Comparison matrix and weighting of level 2 factors in the social group

Criteria	HTC	TQCT	HTKT	GQVL	KNV	Weight (W)
HTC	1.00	3.25	3.34	3.96	4.28	0.44
TQCT	0.31	1.00	0.47	2.90	2.86	0.17
HTKT	0.30	2.15	1.00	2.67	3.18	0.24
GQVL	0.25	0.34	0.37	1.00	2.09	0.09
KNV	0.23	0.35	0.31	0.48	1.00	0.07

From the results of the general comparison of experts in table 6, calculate the same as the level 2 factor belonging to the economic group, using the separate vector method to calculate the weights of the factors, thereby determining the weight vector (table 7) as follows:

[W_{HTC}; W_{TQCT}; W_{HTKT}; W_{GQVL}; W_{KNV}]=[0.44; 0.17; 0.24; 0.09; 0.07]

Regarding the group of ecological environment standards: a matrix comparing 5 evaluation factors is established: Quantity and quality of water (WATER); soil Quality (SOIL); residues of fertilizers and pesticides (DL); biodiversity Reduction (GDSH); incremental disease outbreaks (GTDB) from experts, choose results with CR<10% consistency. The following results:

Table 8. Comparative value of level 2 factors of experts in ecological environment group

Comparision		Order of expert evaluation results									Aij
i	j	1	2	3	4	5	6	7	8	9	
WATER	SOIL	1	2	2	1	1	2	2	5	1	1627/1000
	DL	5	3	3	3	7	5	3	3	5	977/250
	GDSH	3	4	4	7	5	7	5	7	7	5221/1000
	GTDB	5	3	4	7	5	7	7	7	7	1389/250
SOIL	DL	3	2	3	3	5	3	3	2	3	1451/500
	GDSH	5	3	3	5	7	5	3	5	5	2189/500
	GTDB	5	3	5	5	7	5	5	5	5	613/125
DL	GDSH	2	2	3	3	3	5	3	3	3	1451/500
	GTDB	2	3	3	5	5	5	3	3	5	3599/1000
GDSH	GTDB	1/3	1/3	1/2	1/2	1	1	1/3	1/2	1/2	51/100
CR(%)		7.6	7.0	5.4	4.9	8.9	6.4	6.7	6.0	6.7	6.6

From the Aij comparison results of 5 ecological environment factors shown in Table 8, a comparison matrix is established and weighted for these factors (Table 9).

Table 9. Comparison matrix and weighting of level 2 factors belonging to ecological environment group

Criteria	WATER	SOIL	DL	GDSH	GTDB	Weight (W)
WATER	1.00	1.63	3.91	5.22	5.56	0.43
SOIL	0.61	1.00	2.90	4.38	4.90	0.32
DL	0.26	0.34	1.00	2.90	3.60	0.16
GDSH	0.19	0.23	0.34	1.00	0.51	0.06
GTDB	0.18	0.20	0.28	1.96	1.00	0.08

Similar to the above, proceed to apply the separate vector method to calculate the weights of the factors, thereby, determining the weight vector as follows:

$[W_{WATER}; W_{SOIL}; W_{DL}; W_{GDSH}; W_{GTDB}]=[0.43; 0.32; 0.16; 0.06; 0.08]$

As a result, the partial weights of factor levels 1 and 2 have been determined. Continue to calculate the global weights based on these results:

Table 10. Hierarchical structure and weighting of Eco-Sustanability factors

Criterion level 1		Criterion level 2		Weight total
Criteria	W_1	Criteria	W_2	$W_i=W_1*W_2$
1. Economy (KT)	0,59	1. Cost (CP)	0.430	0.254
		2. Profit (LN)	0.250	0.148
		3. Capital efficiency (HQĐV)	0.160	0.094
		4. Productivity (NS)	0.090	0.053

		5. Area (DT)	0.060	0.035
2. Society (XH)	0,11	6. Financial support (HTC)	0.440	0.048
		7. Cultivation practices (TQCT)	0.170	0.019
		8. Technical assistance (HKT)	0.240	0.026
		9. Settlement of jobs (GQVL)	0.090	0.010
		10. The suitability of capital (KNV)	0.070	0.008
3. Eco-environment (MST)	0,20	11. Water quantity and quality (WATER)	0.430	0.086
		12. Soil quality (SOIL)	0.320	0.064
		13. Residues of fertilizers and pesticides (DL)	0.160	0.032
		14. Reducing biodiversity (GDSH)	0.060	0.012
		15. Increasing disease (GTDB)	0.080	0.016

3.2. Assessing the applicability of eco-sustainable criteria to the coffee farming model, the advantages and barriers of the application of the set of criteria

By applying the AHP method for factors at level 1 and level 2, it shows that:

For level 1 factors in Table 10, the economic pressure is the most influential factor on farming (W_{KT}=0.59) of the study area, followed by ecological environment factor (W_{MST}= 0.20) and social factors (W_{XH}=0.11) have the least influence on this process.

For 15 factors at level 2 and total weight shown in Figure 2, there are 6 selected factors that most affect the Eco-Sustanability of coffee production in the specialized farming area of Dak Ha, Kon Tum province, the order is as follows: Cost (CP) > Profit (LN) > Efficiency of capital (HQDV) > Water quantity and quality (WATER) > Soil quantity and quality (SOIL) > Productivity (NS).

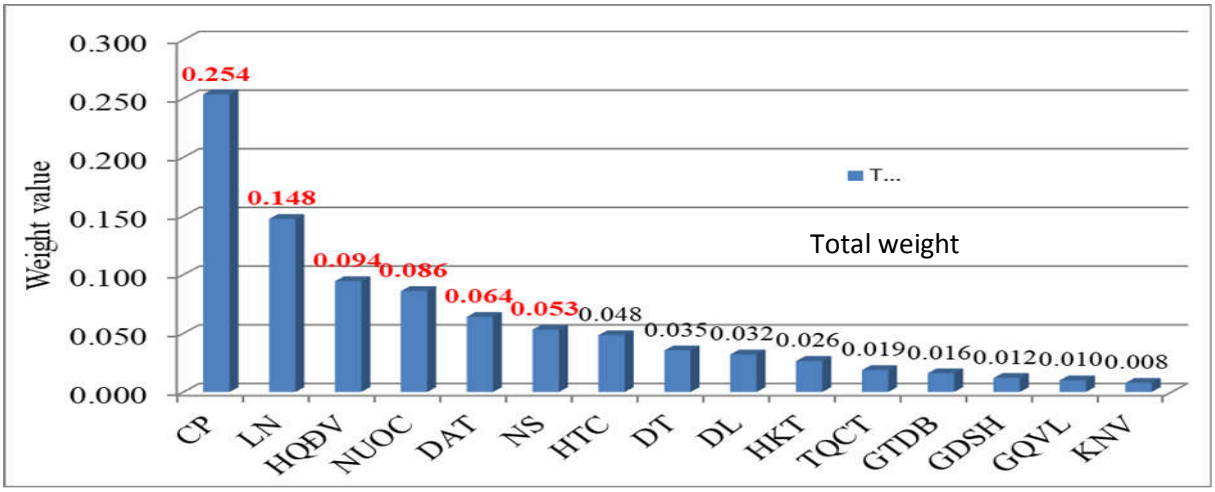

Figure 2. The influence degree of factors on Eco-Sustainability in coffee farming

a) Economic factors

Faced with the trend of increasing social integration and development, farmers are being greatly impacted by economic pressure, a reasonable farming model that is ecologically sustainable while also ensuring increased income. Improving living standards is a problem that people are always interested in and want to be answered in this period.

In Figure 2, the total weight of the influencing factors of economic criteria is as follows: Cost (W=0.254) > Profit (0.148) > Efficiency of capital (0.094) > Productivity (0.053) > Area (0.035). In

which, 4 factors have the most influence on Eco-Sustainability.

In coffee farming, farmers cover the main costs of buying seeds, fertilizers, fuel for watering, pesticides, and labor. Although the conditions of capital that people pay for are different, the survey results show that, on average, the current total investment cost per hectare of coffee is about 62 million VND/ha. Table 11 shows that households paying for irrigation and pesticides per hectare of coffee ranges from 1 to 10 million VND; fertilizer costs are quite high, and more than 60% of households pay from 10 million or more for 1 ha of coffee.

Table 11. Service costs for coffee farming

Main cost	Cost/ha	Percentage of households (%)
Cost of organic fertilizer in business period	<3 million	1.29
	from 3 to less than 10 million VND	31.47
	from 10 million or more	67.24
Cost of chemical fertilizers in business period	<10 million	6.82
	from 10 to less than 20 million VND	26.71
	20 million or more	66.47
Irrigation cost	<4 million	8.04
	from 4 to less than 10 million VND	73.62
	from 10 million or more	18.34
Cost of pesticides	<1 million	1.81
	from 1 to less than 10 million VND	64.46
	from 10 million or more	33.73

According to the situation described above, the first factor that accounts for high costs in coffee farming is fertilizing coffee trees, particularly chemical fertilizers.

b) Regarding ecological environment factors

In the global index, the ecological factors are evaluated in order from highest to lowest, as follows: Water quantity and quality (W=0.086) > Soil quality (0.064) > Residue fertilizers and pesticides (0.032) > Increase in diseases (0.016) > Decrease in biodiversity (0.012). The quantity and quality of water and soil are the most pressing issues affecting the Eco-Sustainability (Figure 2) of the current farming model.

About water supply for irrigation [5] and the assessment of soil suitability for coffee trees in Dak Ha district, Kon Tum province [6] have been presented in the author's previous articles.

c) Regarding social factors

Social factors are one of the three factors that have the least influence on Eco-Sustainability in coffee farming; however, to ensure sustainable development, solving social problems is also a content that requires attention and support ensure long-term viability. The order of the global weight of each factor is as follows: Financial assistance (0.048) > Technical assistance (0.026) > Cultivation practices (0.019) > Job creation (0.010) > Suitability capital capacity (0.008).

As mentioned in the economic factor, coffee farming requires significant investment costs; therefore, people with limited capital require financial assistance to invest. Currently, households are looking for a variety of financial assistance for farming. To ensure output for products and investment funds, 42.2% of households collaborate with cooperatives, coffee purchasing companies, and material supply companies. Furthermore, 100% of households

borrow from banks (55% from state banks, 11% from private banks, and 34% from other states, cooperative, and private support funds).

In the past time, with technical support for farming and harvesting coffee from the state, NGOs and coffee enterprises, 77.7% of households received technical training. The farmers who received training on planting, tending, harvesting, and preliminary processing techniques accounted for the highest percentage (76.3%), technical training on varieties (6.6%), grafting, and improvement (11.8%) and sustainable farming (14.6%) is limited.

4. CONCLUSION

The research identified 15 factors related to economic, social, and environmental issues based on the criteria for assessing the sustainability of agricultural organizations in general and coffee organizations in particular which is divided into 2 functional levels. Through testing and analysis, six factors have the greatest influence on the Eco-Sustainability of coffee production in specialized farming areas, with cost having the greatest influence (W_{CP}=0.254) and productivity having the least (W_{NS}=0.053). Other factors affecting eco-Sustainability in coffee cultivation are respectively profit (W_{LN}=0.148), capital efficiency (W_{HQDV}=0.094), water quantity and quality (W_{WATER} =0.086), and soil quantity and quality (W_{SOIL} =0.064).

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THERMOPHYSICAL PROPERTIES OF SNAKEHEAD FISH (*Channa striata*) MUSCLE CHANGE DURING CHILLING PROCESS

Nguyen Van Minh[1,*], Luong Duc Vu[2]

ABSTRACT

This study was conducted to identify the initial freezing point temperature (t_f) of water and to investigate the changes of thermophysical properties of snakehead fish muscle during the chilling process. The temperatures of snakehead fish muscle during the chilling process and t_f identification decreased from 25.7°C to 0.5°C, and from 28.3°C to -8.2°C, respectively. The result indicated that the initial freezing point temperature (t_f) of water in the snakehead fish muscle was -1.3°C. The temperature of snakehead fish muscle affected considerably the thermophysical properties of snakehead fish muscle. The density (ρ) and specific heat (*c*) increased with decreasing temperature during the chilling process. Inversely, the thermal conductivity (k), thermal diffusivity (α) and enthalpy (h) decreased with decreased temperature. The results obtained from this study could be applied in calculating the energy requirements and equipment used in snakehead fish processing industry.

Keywords: *Chilling, freezing point temperature, snakehead fish, thermophysical property.*

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1. INTRODUCTION

For several decades, aquaculture in the Mekong Delta region of Vietnam has played an important role to the delta communities through improvements in the provision of animal protein, creation of jobs, and generation of income, as well as contribution to the domestic consumption and exports of aquatic products of Vietnam. There is an increasing growth in freshwater aquatic production that includes catfish (Tra and Basa catfish), tilapia, anabas, snakehead fish, and freshwater prawns. The farming area of snakehead fish, especially striped snakehead fish (*Channa striata*), has rapidly increased [1]. Snakehead fish is mainly cultured in small-scale farms with high stocking density and fed by low-value fish or trash fish, which can help reduce poverty [2]. Snakehead fish is considered as a source of high-quality protein because of the fine flesh and flavor, and traditional remedy of sickness [3]. Snakehead fish muscle contains a high protein content (78.32±0.23% dry matter), lipids (2.08±0.08% wet matter), and vitamin A (0.265±0.013 mg/100g fish muscle). It also has high contents of arachidonic acid (AA) 20: 4ω6 (8.2±0.1% total lipid), docosahexaenoic acid (DHA) 22: 6ω3 (13.0±7% total lipid) and eicosapentaenoic acid (EPA) 20: 5ω3 (0.6±0% total lipid) [4]. These components vary depending on species, sex, size, harvest season, feed composition and habitat conditions [5].

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Knowledge of thermophysical properties of foods is thought to be useful in new product development and design of processing equipment, in process calculations of heating and cooling rates of times, and in calculating the process energy requirements as well as in the development of new processing techniques [6]. Thermophysical properties of foods commonly used in calculating heat and mass transfers include density (ρ), specific heat (c), thermal conductivity (k), thermal diffusivity (α) and enthalpy (h) [7]. Thermophysical properties of foods strongly depend upon the chemical composition [8] and temperature [9, 10], thus, using mathematical models which account for the effects of chemical composition and temperature is the most common method used to predict the thermophysical properties of foods [11, 10].

To date, there have been no reports on the changes in the thermophysical properties of snakehead fish muscle during chilling process. Therefore, the objective of this study was to determine the changes in thermophysical properties of snakehead fish muscle during chilling process, including the density (ρ), specific heat (*c*), thermal conductivity (k), thermal diffusivity (*α*) and enthalpy (h). The initial freezing point temperature (t_i) of water in the snakehead fish muscle was also determined.

2. MATERIALS AND METHODS

2.1. Snakehead fish

Snakehead fish used for this research were bought from the local fish farm in Nha Trang city, Khanh Hoa province. The average weight of the fish was 700-800 g. The fish were then transported to the labs of Nha Trang University alive in water containers. Fish were recovered for 2 h before bleeding and gutting in following the procedure described in Nguyen *et al.* (2021) [12].

2.2. Chilling process

The gutted snakehead fish were chilled using slurry ice (crushed ice : water ratio of 1 : 1) in Styrofoam boxes at a ratio of fish to slurry ice of 1 : 2 (w/w). The changes in temperatures of fish muscle were recorded using a 12-channel Thermocouple (Data logger TM500, Extech Instruments, Boston, Massachusetts, USA). Three sensors were inserted into the fish muscle by piercing the loin to varying depths with a 12-gauge stainless steel needle. The thermocouple wire was inserted into the holes; one in the skin, one beneath the skin and another one in the center of the dorsal muscle to indicate the rate of cooling (temperature reduction) in different geometrical locations in the fish body.

2.3. Initial freezing point temperature determination

The initial freezing point temperature of water in the snakehead fish muscle was determined by the freezing curve method described by Rahman *et al.* (2002) [13]. Snakehead fish samples were frozen in a freezer at temperature of -20°C (Sanaky VH-6699W3). The temperature-time of snakehead fish muscle was recorded by using a 12-channel Thermocouple. The freezing point was derived from the time-temperature data plot.

2.4. Thermophysical properties determination

The thermophysical properties of snakehead fish muscle including density (ρ), specific heat (*c*), thermal conductivity (k), thermal diffusivity (α) and enthalpy (h) were measured according to the mathematical models developed by Choi and Okos (1986) [8] (Table 1).

Table 1. Mathematical models for thermophysical properties determinations

Thermophysical property	Food Component	Thermophysical property model
Density (ρ), (kg/m^3)	Protein	$\rho = 1.3299 \times 10^3 - 5.1840 \times 10^{-1} \times t$
	Fat	$\rho = 9.2559 \times 10^2 - 4.1757 \times 10^{-1} \times t$
	Carbohydrate	$\rho = 1.5991 \times 10^3 - 3.1046 \times 10^{-1} \times t$
	Fiber	$\rho = 1.3115 \times 10^3 - 3.6589 \times 10^{-1} \times t$
	Ash	$\rho = 2.4238 \times 10^3 - 2.8063 \times 10^{-1} \times t$
	Water	$\rho = 9.9718 \times 10^2 + 3.1439 \times 10^{-3} \times t - 3.7574 \times 10^{-3} \times t^2$
	Total	$\rho = \frac{(1-\varepsilon)}{\frac{\sum x_i}{\rho_i}}$
Specific heat (c), kJ/kg.K	Protein	$C_P = 2.0082 + 1.2089 \times 10^{-3} \times t - 1.3129 \times 10^{-6} \times t^2$
	Fat	$C_P = 1.9842 + 1.4733 \times 10^{-3} \times t - 4.8008 \times 10^{-6} \times t^2$
	Carbohydrate	$C_P = 1.5488 + 1.9625 \times 10^{-3} \times t - 5.9399 \times 10^{-6} \times t^2$
	Fiber	$C_P = 1.8459 + 1.8206 \times 10^{-3} \times t - 4.6509 \times 10^{-6} \times t^2$
	Ash	$C_P = 1.0926 + 1.8896 \times 10^{-3} \times t - 3.6817 \times 10^{-6} \times t^2$
	Water	$C_p = 4.1289 - 9.0864 \times 10^{-5} \times t + 5.4731 \times 10^{-6} \times t^2$
	Total	$C = \sum C_i \times X_i$
Thermal conductivity (k), W/m.K	Protein	$k = 0.17881 + 1.1958 \times 10^{-3} \times t - 2.7178 \times 10^{-6} \times t^2$
	Fat	$k = 0.18071 - 2.7604 \times 10^{-3} \times t - 1.7749 \times 10^{-7} \times t^2$
	Carbohydrate	$k = 0.20141 + 1.3874 \times 10^{-3} \times t - 4.3312 \times 10^{-6} \times t^2$
	Fiber	$k = 0.18331 + 1.2497 \times 10^{-3} \times t - 3.1683 \times 10^{-6} \times t^2$
	Ash	$k = 0.32962 + 1.4011 \times 10^{-3} \times t - 2.9069 \times 10^{-6} \times t^2$
	Water	$k = 0.57109 + 1.7625 \times 10^{-3} \times t - 6.7036 \times 10^{-6} \times t^2$
	Total	$k = \sum x_i^V . ki$
Thermal diffusivity (α), m^2/s	Protein	$\alpha = 6.8714 \times 10^{-8} + 4.7578 \times 10^{-10} \times t - 1.4646 \times 10^{-12} \times t^2$
	Fat	$\alpha = 9.8777 \times 10^{-8} - 1.2569 \times 10^{-11} \times t - 3.8286 \times 10^{-14} \times t^2$
	Carbohydrate	$\alpha = 8.0842 \times 10^{-8} + 5.3052 \times 10^{-10} \times t - 2.3218 \times 10^{-12} \times t^2$
	Fiber	$\alpha = 7.3976 \times 10^{-8} + 5.1902 \times 10^{-10} \times t - 2.2202 \times 10^{-12} \times t^2$
	Ash	$\alpha = 1.2461 \times 10^{-8} + 3.7321 \times 10^{-10} \times t - 1.2244 \times 10^{-12} \times t^2$
	Water	$\alpha = 1.3168 \times 10^{-7} + 6.2477 \times 10^{-10} \times t + 2.4022 \times 10^{-12} \times t^2$
	Total	$\alpha = \sum \alpha_i . X_i^V$
Enthalpy (h), kJ/kg	Unfrozen water	$h_f = 9.79246 + 405.096 \times x_w$
		$h = H_f + (t - t_f) \times (4.19 - 2.3 \times x_S - 0.628 \times x_s^3)$
		$h = \sum H_i . X_i = \sum \int_{t1}^{t2} C_i . X_i \, dT$

2.5. Proximate analysis

Proximate analysis was performed according to the AOAC (2000) [14] standard methods. Moisture and ash contents were determined using the AOAC method 976.05 and 942.05, respectively. The protein content was determined using nitrogen determination by the Kjeldahl method from AOAC method 954.01. The conversion factor used to calculate crude protein content was 6.25. To determine the lipid content of the samples, Bligh and Dyer (1959) [15] method was used.

2.6. Statistical analysis

Data analyses were done using Microsoft Excel 2019 (Microsoft Inc. Redmond, Wash.,

USA). One way analysis of variance (ANOVA), Duncan's test (Post-hoc) and Pearson correlation analysis were performed on means of the variable values in the statistical program NCSS 2000 (NCSS, Kaysville, Utah, USA). The significance of differences was defined at the 5% level ($p<0.05$).

3. RESULTS AND DISCUSSION

3.1. Chemical composition of fresh snakehead fish muscle

Table 2. The chemical compositions of fresh snakehead fish muscle

Component	Value
Water (%WB)	77.6±0.13
Protein (%WB)	18.2±0.24
Lipid (%WB)	2.71±0.12
Ash (%WB)	1.09±0.02
Carbohydrate (%WB)	0.34±0.03

The chemical compositions of fresh snakehead fish muscle are presented in Table 2. Moisture, protein, lipid, ash, and carbohydrate in snakehead fish (*Channa striata*) muscle were of 77.6±0.13%, 18.2±0.24%, 2.71±0.12%, 1.09±0.02% and 0.34±0.03%, respectively.

The results revealed that snakehead fish is a medium-fatty fish species. Osman *et al.* (2001) [16] reported that low-fat fish have higher water content, as observed in this study. The proximate composition of the snakehead fish reported by Chedoloh *et al.* (2011) [4], Rahman *et al.* (2018) [17] and by Zuraini *et al.* (2006) [18] showed some degrees of differences, especially for the fat content. Such variations in the chemical composition of fish are closely depended on the age, size, sex, environmental conditions and season [19]. The farmed fish generally have a higher lipid content compared to wild fish [20].

3.2. Freezing point temperature of water in the snakehead fish muscle

Initial freezing point temperature is one of the most important properties of food that is used in prediction models of thermal properties, freezing and thawing times in order to optimize the process and the product quality. It is also used for determining the food's proper processing and storage conditions [13]. The initial freezing point of water in the snakehead fish muscle was determined using the freezing curve method. From the time-temperature data plot (Figure 1), the initial freezing point temperature of water in the snakehead fish muscle was practically found to be -1.3°C (point B) and the undercooled temperature was -2.8°C (point A). The initial freezing point temperatures of water in the fish muscle are different from different species, mainly due to the disparity between methods applied, and variation of fish species in terms of chemical composition and freezing point depressants. However, the result from this study was found to be correlated with the initial freezing point temperature of -1.4°C for long tail tuna (*Thunnus tongol*) [21] and -1.5°C for salmon (*Salmo salar*) [22].

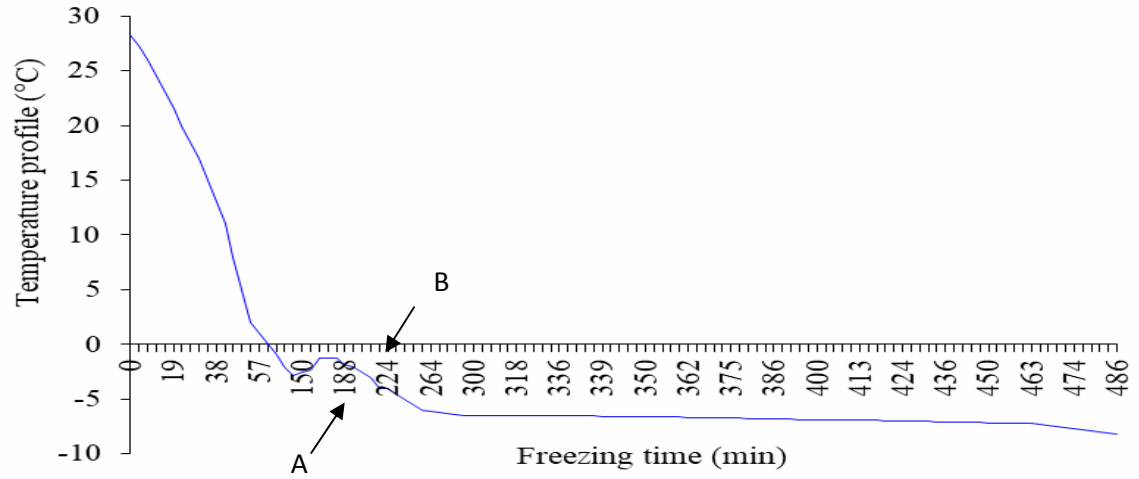

Figure 1. The initial freezing point temperature of water in the snakehead fish muscles

3.3. Changes in density (ρ) of snakehead fish during chilling process

Although the mass density of the fish is not involved with the group of thermal properties, however, it was determined along with the other properties due to its significance in heat transfer calculation. The changes in density of snakehead fish as a function of temperature are presented in Figure 2. It is obvious to see that the density increased slightly (from 1,042 kg/m^3 to 1,046 kg/m^3) with decreasing temperature over this range of temperature applied in the chilling process. It is well-known that the fish muscle is composed mainly of water, therefore, the behavior of water constituent will dominate the density behavior. The results of this study were in agreement with those published by Abbas *et al.* (2009) [23] for Malaysian yellowtail catfish.

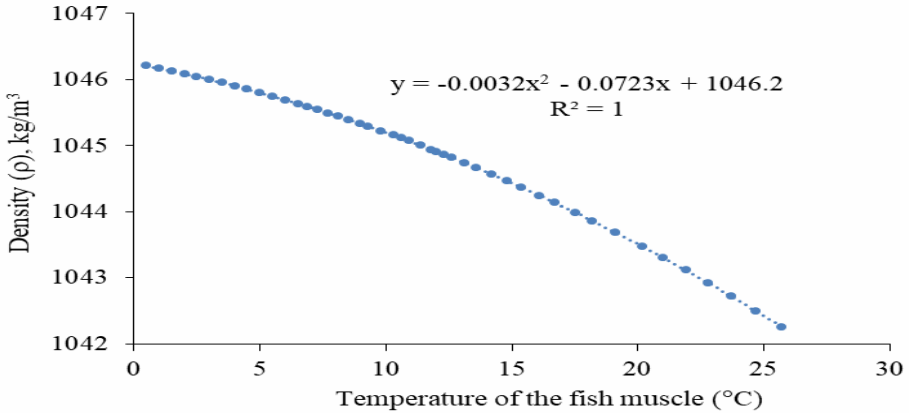

Figure 2. Variation of the density of snakehead fish muscle with temperature

3.4. Changes in specific heat (c) of snakehead fish during chilling process

Specific heat is a measure of the energy required to change the temperature of a kg of food by one degree. Therefore, the specific heat of foods can be used to calculate the heat load imposed on the refrigeration equipment by the cooling or freezing of foods. Generally, the specific heat of snakehead fish muscle increased slightly from 3.653 kJ/kg.K to 3.662 kJ/kg.K during the chilling process (Figure 3). The pattern of changes in specific heat agreed with that noted by Kreith and Bohn (1993) [24]. The results from the present study were in accordance with those published by Radhakrishnan (1997) [25] for bluefish, salmon, seabass, tilapia, trout and tuna. The author reported the specific heat of these fish species ranged from 3.1 kJ/kg.K to 3.8 kJ/kg.K. The specific heat of fresh seafood at a temperature of 17°C varied from 3.29 kJ/kg.K to 3.79 kJ/kg.K [26].

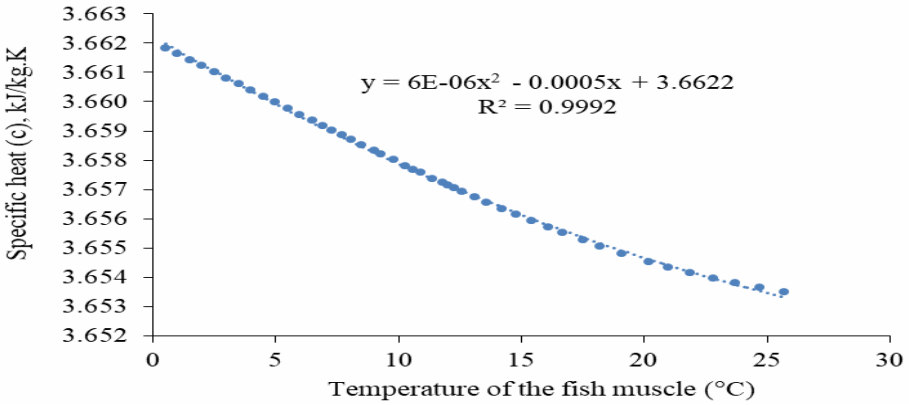

Figure 3. Changes in specific heat of snakehead fish muscle during chilling process

3.5. Changes in thermal conductivity (k) of snakehead fish during chilling process

Thermal conductivity (k) measures the heat conducting ability of a material. Materials with low thermal conductivity have a lower rate of heat transfer compared to materials with high thermal conductivity. The thermal conductivity of snakehead fish muscle decreased linearly with decreasing temperature of the fish muscle over the range of experimental temperature with R^2 = 0.9997 (Figure 4). The thermal conductivity of snakehead fish muscle in general ranged from 0.498 W/m.K to 0.539 W/m.K at temperatures 0.5 to 25.7°C. Kumbhar *et al.* (1981) [27] reported that the k values of mackerel were in the range of 0.416 to 0.459 W/m.K at temperatures 0 to 30°C. The k value of 0.502 W/m.K was reported for salmon (*Salmo salar*) at a temperature of 4°C. Radhakrishnan (1997) [25] demonstrated the k values for bluefish ranged from to 0.424 to 0.628 W/m.K with a temperature ranging from 6.7 to 29.47°C and for tilapia the k values ranged from 0.442 to 0.548 W/m.K. The thermal conductivity of Tuna (*Katsuwonus pelamis*) of 0.57±0.01 W/m.K at ambient temperature and 71.6% moisture content. It is well-documented that the thermal conductivity is much dependent on the temperature and chemical composition of food muscle [11, 28]. In general, it could be concluded that the thermal conductivity of snakehead fish muscle agreed well with the values found in the literatures for fish.

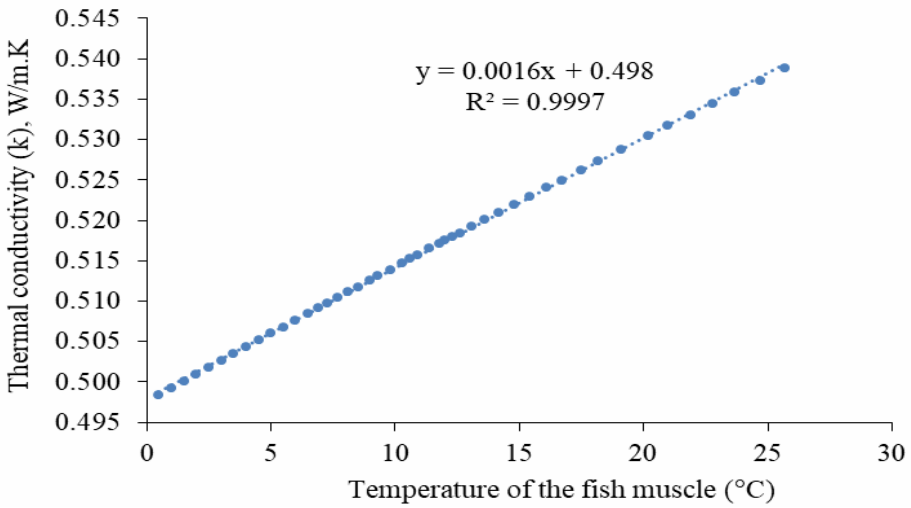

Figure 4. Thermal conductivity changes during chilling snakehead fish as a function of temperature

3.6. Changes in thermal diffusivity (α) of snakehead fish during chilling process

Thermal diffusivity (α) is a physical property associated with transient heat flow. It measures the ability of a material to conduct thermal energy relative to its ability to store thermal energy. Materials with large thermal diffusivity will respond quickly to changes in their thermal environment while materials of small thermal diffusivity will respond more slowly, taking longer to reach a new equilibrium condition. In this study, the thermal diffusivity of snakehead fish muscle decreased from 1.35×10^{-7} m^2/s to 1.22×10^{-7} m^2/s during the chilling process (temperature of the fish muscle decreased from 25.7°C to 0.5°C) (Figure 5). The α values obtained for snakehead fish muscle were in the ranges reported for bluefish, salmon, seabass, tilapia, trout and tuna by Radhakrishnan (1997) [25]. Kumbhar *et al.* (1981) [27] also reported the thermal diffusivity values for 12 different fish species were in the range of 1.086×10^{-7} m^2/s to 1.875×10^{-7} m^2/s.

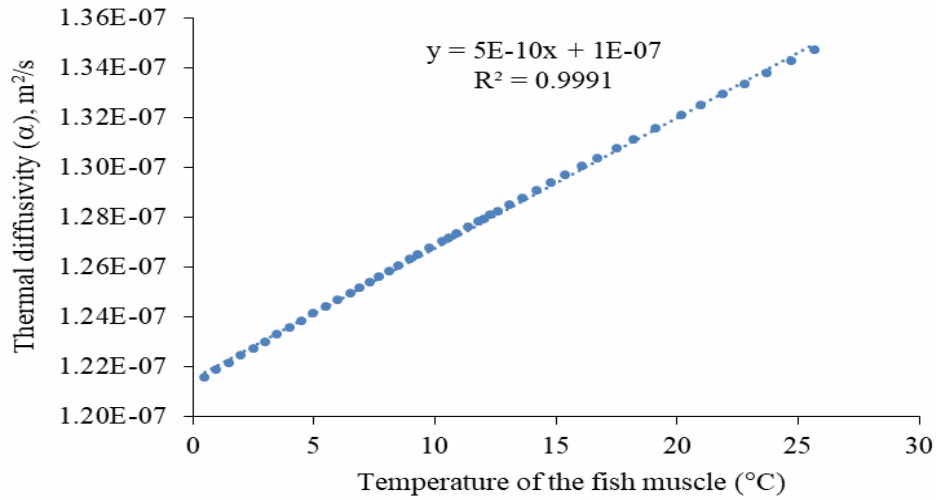

Figure 5. Changes in thermal diffusivity of snakehead fish muscle as a function of temperature

3.7. Changes in enthalpy (h) of snakehead fish during chilling process

The change in a food's enthalpy can be used to estimate the energy that must be added or removed to affect a temperature change. It is obvious to see that the enthalpy of snakehead fish muscle was a function of temperature. The enthalpy decreased well-linearly (R^2 = 1.00, from 423.36 kJ/kg to 330.91 kJ/kg) with decreasing temperature during the chilling process (Figure 6). The trend of enthalpy variation obtained from the present work was in agreement with that reported by Hobani and Elansari (2008) for four different types of meat. However, the results showed some degrees of differences. The enthalpy values ranged from 291.36 kJ/kg to 428.50 kJ/kg for Noemi meat at 40°C reported by Hobani and Elansari [29]. The differences might be due to the differences in chemical composition of the samples and different temperatures used for enthalpy measurements.

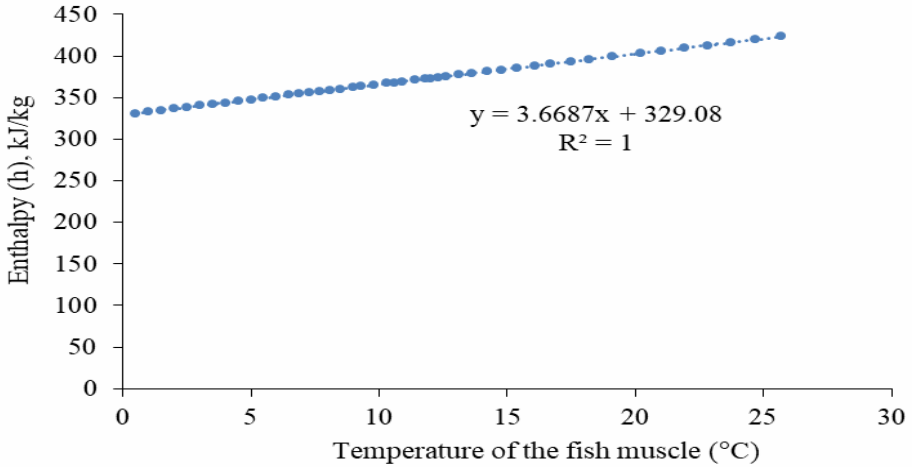

Figure 6. Changes in enthalpy of snakehead fish muscle during chilling process

4. CONCLUSION

The initial freezing point temperature of water in the snakehead fish muscle was -1.3°C. Thermophysical properties of snakehead fish muscle depended on the temperature of the fish muscle during chilling process. The density (ρ) and specific heat (*c*) increased from 1,042 kg/m^3 to 1,046 kg/m^3 and from 3.653 kJ/kg.K to 3.662 kJ/kg.K, respectively when the temperature of the fish muscle decreased from 25.7°C to 0.5°C.

Inversely, the thermal conductivity (k), thermal diffusivity (α) and enthalpy (h) decreased from 0.539 W/m.K to 0.498 W/m.K, from 1.35×10^{-7} m^2/s to 1.22×10^{-7} m^2/s and from 423.36 kJ/kg to 330.91 kJ/kg, respectively. The results obtained from this study could be applied in snakehead processing industry.

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POTENTIAL USE OF *Scirpus littoralis* AND *Typha orientalis* FOR NITROGEN AND PHOSPHORUS REMOVAL FROM INTENSIVE WHITELEG SHRIMP WASTEWATER

Ngo Thuy Diem Trang[1,*]

ABSTRACT

The rapid development of whiteleg shrimp farming in the Vietnamese Mekong Delta has an adverse impact on the environment due to large amount of nitrogen and phosphorus content in wastewater and pond sludge/sediment. Phytoremediation is a promising technique to use plant for mitimigating environmental impacts from intensively whiteleg shrimp culture. Selection of potential plant species having fast growth, high biomass, high adaptive and nutrient uptake capacity in wetlands condition is one of the steps for the success of this technique. Growth responses, nitrogen and phosphorus removal process were investigated at three water levels of +15 cm, +30 cm and +45 cm with plants (*Typha orientalis* and *Scirpus littoralis*) and without plants (as control treatment). They were arranged in a completely randomized design with three replications. The plants were supplied wastewater from intensive whiteleg shrimp tanks once every two weeks. Waterlogged assessment was conducted for 71 days. Water levels significantly affected plant growth rate and nutrient removal capacity. *S. littoralis* grew well with a lower survival rate and had no statistical reduction of biomass compared to *T. orientalis* at the highest water level of +45 cm. *T. orientalis* had the best perfromance in growth and biomass responses to waterlogged at water levels of +15 cm and +30 cm. The results indicated that *S. littoralis* was the best of choice to grow in waterlogged condition of the shrimp pond for maintaining water quality.

Keywords: *Aquaculture effluent, plant biomass, Litopenaeus vannamei, water levels, waterlogged.*

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1. INTRODUCTION

Total area and production of shrimp farming in Vietnam in 2021 was 752,900 ha and 996,269 tonnes, in which the Vietnames Mekong Delta (VMD), South of Vietnam, accounted for over 92% and 84% of Vietnamese shrimp farming area and production [1]. Specifically, whiteleg shrimp (*Litopenaeus vannamei*) has increased rapidly in production in recent years, accounting for 67% of total shrimp production from 16% of the total farming area of the country. Anh *et al.* (2010) [2] reported that to produce 1 ton of shrimp 5,345-7,151 m^3 wastewater, 259 kg BOD, 769 kg COD, 1170 kg TSS, 30 kg N, 3.7 kg P and 4.8 kg $N\text{-}NH_3$ discharged into the environment. Shrimp aquaculture growth in Vietnam has suffered many problems which similar to Asia' situation in recent years. The major factors contributing to the problem in sustaining shrimp aquaculture are disease outbreaks, environmental degradation and poor management practice [3]. Poor water quality from the river that is one of the causes in failure of 3,081 ha whiteleg shrimp [1]. Therefore, there is an urgent need to develop a more sustainable aquaculture industry that uses less water, and that

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does not deteriorate the water quality of the rivers. Phytoremediation is defined as the use of plants and their associated microbes for environmental clean-up [4]. One of the phytoremediation processes, in which plants are used to remove contaminants from soils or water into harvestable plant biomass, is called phytoextraction. Phytoextraction is used mainly for extracting heavy metals from polluted soils and water [5], but the use of plant uptake and plant-mediated conversions also has great potential for the removal of nutrients from nutrient enriched waters.

In the present study, *Scirpus littoralis* Schrab and *Typha orientalis* C. Presl were chose to study waterlogged stress. In fact, *T. orientalis* and *S. littoralis* are cultivated in the paddy field and/or in the shrimp ponds in the MD for human food and providing habitat for shrimp, respectively. That is the reason to evaluate flooded level is suitable for plant growth. According to farmers, the purpose of planting sedge is to improve the pond environment and to increase sources of organic feed materials as well as density of natural food in the pond. In addition, the sedge grass helps regulate pond temperature in hot weather. Trang *et al.* (2018) [6] reported that between two studied plants, *T. orientalis* accumulated a higher Na^+ and Cl^- concentration in the roots and shoots especially at the high levels of NaCl of 15-30‰ and *T. orientalis* has lower salinity tolerant capacity compared to S. *littoralis*. They also suggested that both plants are able to withstand salt stress and can be considered the best bio-filter candidate in the integration of constructed wetlands and marine shrimp culture towards sustainable aquaculture in the Mekong delta, Vietnam. To understand the potential effects of increased water levels on these aquatic macrophytes, information is needed regarding the duration and water regime exposure in relation to the response of the plant species in term of growth, biomass and nutrient removal. Therefore, the present study was carried out to identify waterlogged-tolerant species through survival, growth, biomass and nitrogen (N) and phosphorus (P) removal.

2. MATERIALS AND METHODS

2.1. Experimental setup

A completely randomized design consisting of two plant species (*Scirpus littoralis* and *Typha orientalis*) and three water levels (+15, +30 and +45 cm) was set up in triplicates. The corresponding unplanted containers were filled with three water levels of +15, +30 and +45 cm which were considered as control treatments. The experiment was conducted in the greenhouse at the College of Environment and Natural Resources of Can Tho University, Vietnam (10.03° N latitude and 105.76° E longitude).

2.2. Plant materials and growth conditions

Rhizomes and young plants of the two studied species were collected from the fields. Twenty-seven plastic containers of each size of 50-L, 45-L and 120-L were used. These corresponding containers were filled with a 25-L, 30-L and 80-L of wastewater that made up water levels at +15, +30 and +45 cm above sediment. Sixteen kg of sediment (76.2% dry weight) which was collected from the intensive whiteleg shrimp pond in Soc Trang province were filled in the growth containers to mimic the bottom pond condition.

A valve connected with Ø 34 mm PVC tube was fitted through a hole in the side of the containers at the middle of water level to sample water (Figure 1). The individual young and similar size plants for each species were placed in each container for acclimation of two months before flooding. Then plants were thinned out to keep a density of 15 plants/m^2 [7]. Intensive whiteleg shrimp wastewater collected from the culture tanks, which were stocked at 400 shrimps per one m^3 water were used as growth solution for plants.

2.3. Plant growth and biomass measurement

At the initiation of the flooding treatments, the representative specimens of each container were sampled to determine their initial shoot height, root length, number of shoots, fresh and dry weight. At harvest, plants were carefully removed from the sediment and the roots were carefully washed to remove particle and sediment. The fractions were weighed for fresh biomass and dried at 60°C until constant weight to determine for dry weight.

The shoot and root growth rate were calculated from the difference of shoot height and root length measurements at the beginning and at the end of the study divided by time (a total of waterlogged perios was 71 days). Relative growth rate of biomass was calculated from the difference of natural logarithm of harvested biomass and beginning biomass divided by time.

2.4. Water analysis

Water samples were taken every two weeks at 6:00 - 7:00 AM. Temperature (°C), dissolved oxygen (DO, mg/L), pH, salinity (‰), electrical conductivity (EC, mS/cm), redox potential (E_h, mV) of the water in the containers were measured at the experimental site using respective portable meters. Water samples were collected and transferred immediately to the laboratory for analysis of alkalinity ($CaCO_3$), Ammonium nitrogen (NH_4-N), Nitrite nitrogen (NO_2-N), Nitrate nitrogen (NO_3-N), total Kjeldahl nitrogen (TKN), orthophosphate (PO_4-P) and total phosphorus (TP). All the analytical measurements were carried out according to Standard Methods [8].

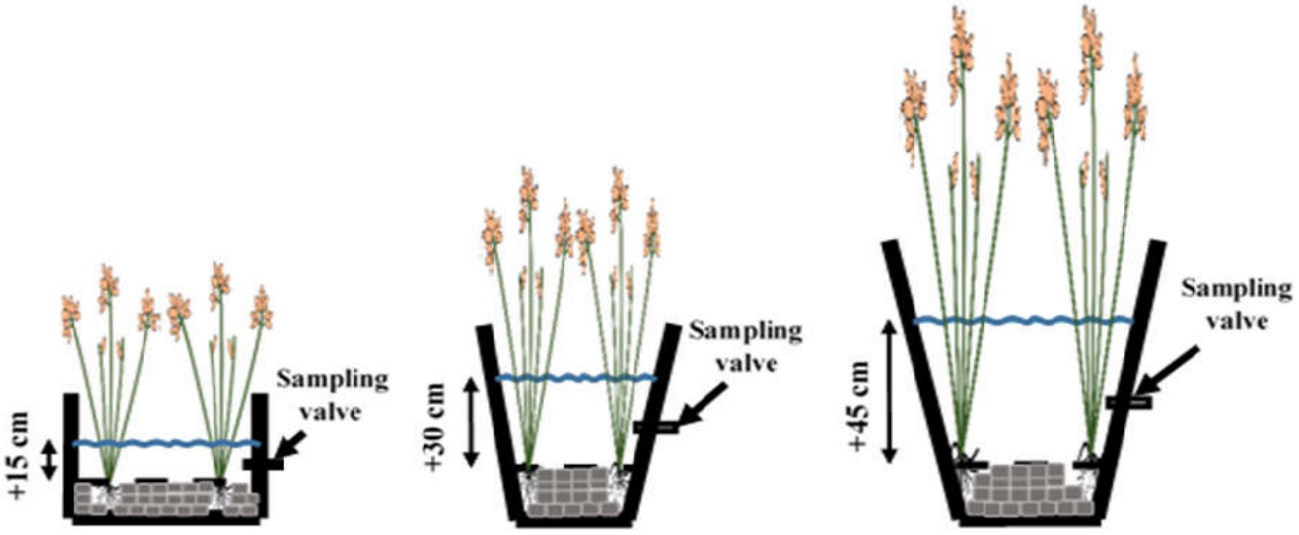

Figure 1. An overview of treatment plots which represented one plant species for three water levels of +15, +30 and +45 cm above sediment

2.5. Statistical analysis

Data were tested for normal distribution, variance homogeneity (Levene's test) and logarithmically transformed if necessary. Differences in water quality were identified by two-way repeated measures ANOVA (General Linear Models) with factor A was plant types (two plants and one unplanted treatment); factor B was water levels (3 levels) which was nested in factor A and factor C was sampling times (6 times) using Type III sum of squares. Differences in plant growth and biomass were identified by one-way ANOVA. Tukey Honestly Significant Differences (HSD) was used to compare significant differences between treatments at the 5% probability level. The software

Statgraphics Centurion XV (StatPoint, Inc., USA) was used for all statistical analyses.

3. RESULTS AND DISCUSSION

3.1. Plant growth and biomass allocation

Water levels significantly affected plant survival rate, number of new shoot and root length ($p<0.05$; Figure 2A, 2B, 2C), but did not affect plant height and growth rate of shoot and root ($p>0.05$; Figure 2). The same fingdings in *Typha domingensis* when the plants were flooded at 40-137 cm depth for six weeks [9]. *T. orientalis* in the water level of +45 cm showed stress symptom of leaf rolling and wilting at the 6th week of waterlogged, and the major part of shoots eventually dried out at the 10th week of waterlogged. Therefore, at the harvest we counted the number of survival plant for *T. orientalis* in the water level of +45 cm which was zero due to no green leaves, but number of new shoot and biomass of those plants was collected and measured. *S. littoralis* had an average of 21% mortality at the water level of +45 cm (Figure 2A). Although two tested species have been known as wetlands plants and they were grown in the commercial extensive shrimp pond in the VMD, they both showed stress of waterlogged at the water level of +45 cm. It can be explained that the growth condition with sallow sediment layer with low nutrient concentration in the sediment and in the wastewater, which might cause unfavourable condition for plant growth [10]. Number of new shoots of *S. littoralis* was higher than that of *T. orientalis* in all water levels and it was also reduced at the highest water level of +45 cm ($p<0.05$). The root length of both species tended to be shortened at the highest water level of +45 cm ($p<0.05$). Between two tested species *S. littoralis* showed more tolerant to waterlogged condition in term of survival. Grace (1989) [11] and Newman *et al.* (1996) [10] reported that nutrient enrichment and shallow water depths (i.e., 20 - 30 cm) were usually favor the dominance of *Typha* species in wetlands. *Typha latifolia* and *T. domingensis* can grow at a wide range of water depths (0 - 115 cm), these species have high shoot density and flowering incidence only within a narrow range of water depths [11]. As water depth increases, *T. domingensis* increases its shoot height, produces fewer but larger ramets, and decreases the incidence of flowering. Increasing water depth reduces the anchorage capacity of plants by decreasing biomass allocation to rhizomes and roots [11].

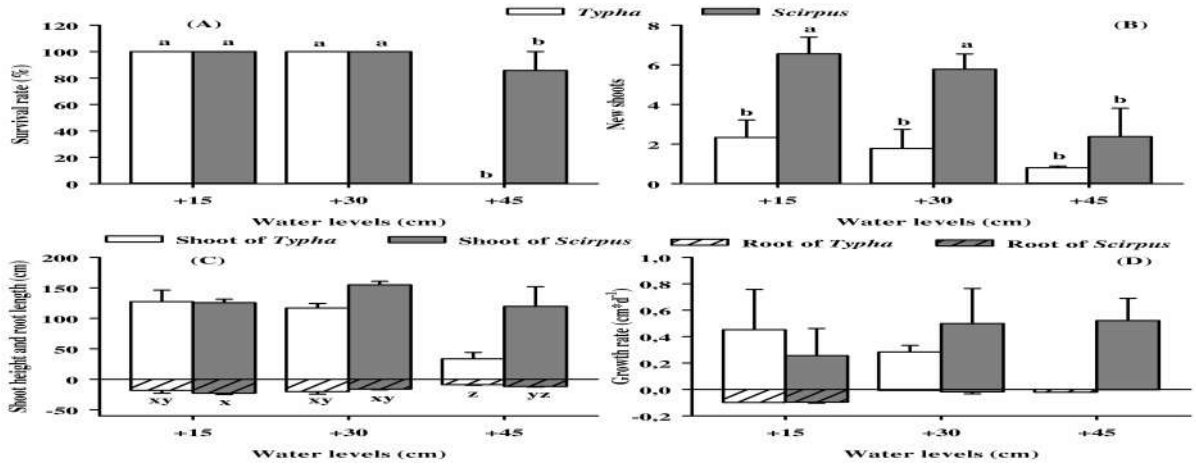

Figure 2. Survival rate (A), number of new shoot (B), shoot height and root length (C) and growth rate of shoot and root (D) of *Typha orientalis* and *Scirpus littoralis* grown at different water levels

Notes: Values are the means of 3 replicates ± S.D. Different letter ^{a,b,x,y} indicates significant difference based on a Tukey HSD test ($p<0.05$).

Water levels significantly affected shoot and root dry and fresh weight at the harvest of the two species ($p<0.05$; Figure 3A & 3B) but did not affect relative growth rate (RGR) of their biomass ($p>0.05$; Figure. 3C & 3D). The species also influenced fresh and dry weight of the shoots and roots. Although the biomass was reduced in both species in response to water levels ($p<0.05$; Figure. 3), it nevertheless was significantly greater in *T. orientalis* than *S. littoralis* under +30 cm water levels ($p<0.05$; Figure 3A & 3B). *S. littoralis* showed no statistical reduction in fresh and dry weight of the shoot and root's fractions across water levels, while *T. orientalis* had a reduction of these parameters at the highest water level of +45 cm.

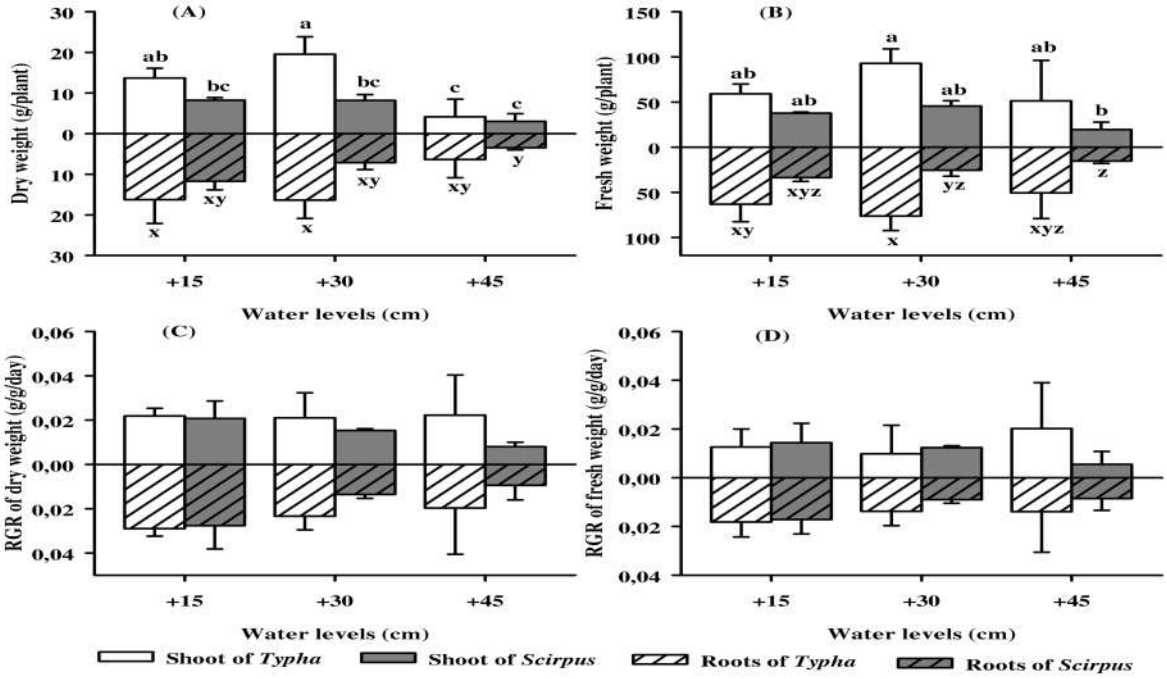

Figure 3. Dry weight (A), fresh weight (B), relative growth rate (RGR) of dry weight (C) and fresh weight (D) of *T. orientalis* and *S. littoralis* grown at different water levels.

Notes: Values are the means of 3 replicates ± S.D. Different letter ^{a,b} indicates significant difference between treatment in the shoot fractions based on a Tukey HSD test ($p<0.05$). Different letter ^{x,y} indicates significant difference between treatment in the root fractions based on a Tukey HSD test ($p<0.05$).

3.2. Water quality

3.2.1. Values of dissolved oxygen, redox potential, temperature and chemical oxygen demand in the water

There were significant differences for dissolved oxygen (DO), redox potential (E_h), temperature and chemical oxygen demand (COD) in the water between treatments and between sampling times ($p<0.05$; Figure 4). Dissolved oxygen is the most critical water quality for shrimp growth. The DO concentrations tended to increase over time ($p<0.05$; Figure 4A) and was at the lowest value in the containers planted *T. orientalis* at the water level of +45 cm ($p<0.05$; Figure 4A). Although the water DO concentration was the lowest in the treatment of *T. orientalis* at +45 cm water level but it was higher than that of the permitted limit (≥3.5) for requirement on shrimp rearing water quality regarding to Appendix 1 of the Vietnamese standards No. 02-19/2014/BNNPTNT (issued on July 29, 2014) [12]. Redox potential is inextricably linked to

oxygen supply and the processes of consumption thereof by microorganisms and plant roots. Therefore, the redox potential is used as an indicator of the oxygenation status and the content of biogenic forms and toxins in the soil environment and sediment. In the case of submerged sediment in our study, penetration of atmospheric oxygen into the sediment was limited due to low rates of oxygen diffusion and, hence, low redox potential, which inhibits plant growth through inhibition of respiration and production of toxins in reducing conditions. Therefore, the reported water redox potential values had similar trend to DO concentration, it increased over time and had the highest values at the end of the study ($p<0.05$; Figure 4B). The average values of E_h in the water of the treatments were in the range of -101 to -75 mV and were classified as reduced environment [13]. The average water temparature of the treatments were in the range of 30.3 - 31.4°C (Figure 4C) which was in the permitted limit (18.0-33.0°C) for requirement on shrimp rearing water quality [12]. Chemical oxygen demand (COD) was not listed in the former Circular No. 45/2010/TT-BNNPTNT [14] for permitted limit in water quality for shrimp growth or in discharged water; however, in the updated Vietnamese standards No. 02-19/2014/BNNPTNT [12] COD parameter was placed in the permitted limit for discharge effluent after treatment of wastewater (≤150 mg/L). In our study, COD concentrations in all the treatments fluctuated and tended to increase at the end of the study, but they were 6-times lower than that of the permitted limit. In sum, DO, temperature and COD concentrations in the water across the treatments meet the Vietnamese standards requirement on shrimp rearing water quality. The water in the treatments was qualified and can be reused for rearing shrimp.

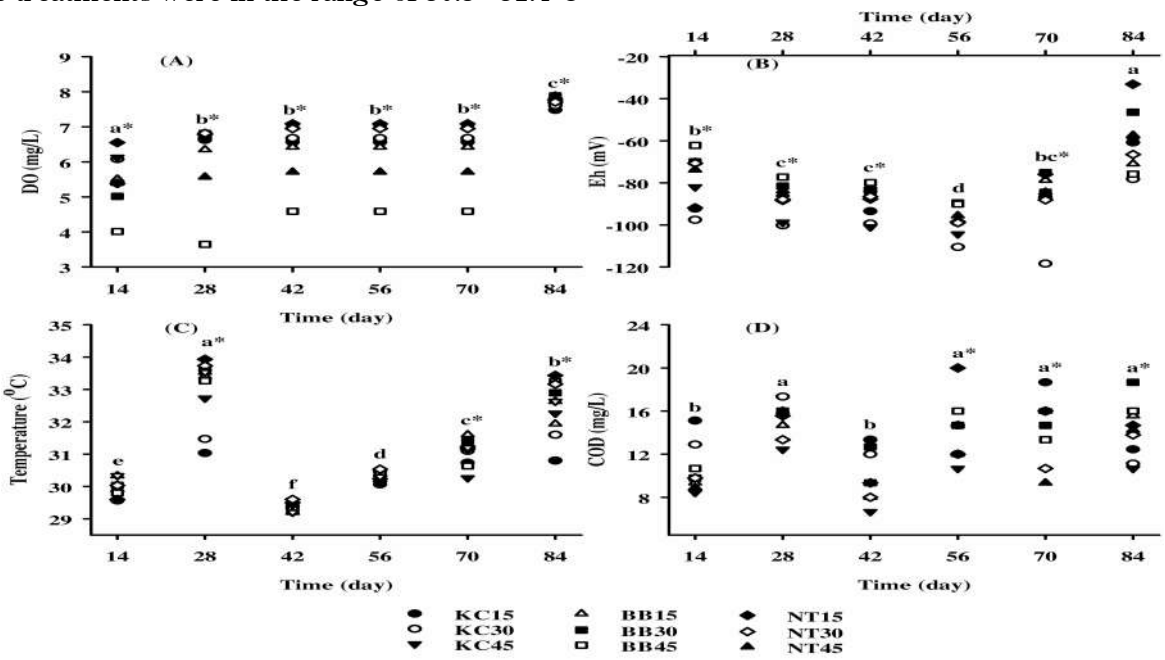

Figure 4. Dissolved oxygen (A), redox potential (B), temperature (C) and chemical oxygen demand (D) in the water over time of *T. orientalis* (BB), *S. littoralis* (NT) and unplanted (KC) at +15, +30 and +45 cm water levels

Notes: Values are the means of 3 replicates. Asterisk (*) indicates significant difference between treatments within sampling time. Different letter [a,b,c] indicates significant difference between sampling times based on a Tukey HSD test ($p<0.05$).

3.2.2. Values of pH, electrical conductivity, salinity and alkalinity in the water

The pH values can affect to the physicaly, chemically, biological elements of the environment and shrimp health. In particular, an increase of pH and temperature values is the cause of increase of NH_3-N concentration that can toxic to the aquatic organisms. During the experiment, the average of water pH in the treatments maintained between 7.9 and 8.4 (Figure 5A) that was in the optimum level for shrimp growth regarding to Appendix 1 of the Circular No. 45/2010/TT-BNNPTNT (7.5-8.5) [14] and in the permitted limit (7.0-9.0) for requirement on shrimp rearing water quality [12]. The pH values had similar trend with DO concentrations in the treatment without plants which was higher than that of the other planted treatments ($p<0.05$; Figure 5A). Similar to DO, pH values were also related to algae photosynthesis because algae bloomed (via visual observation) in the unplanted treatments.

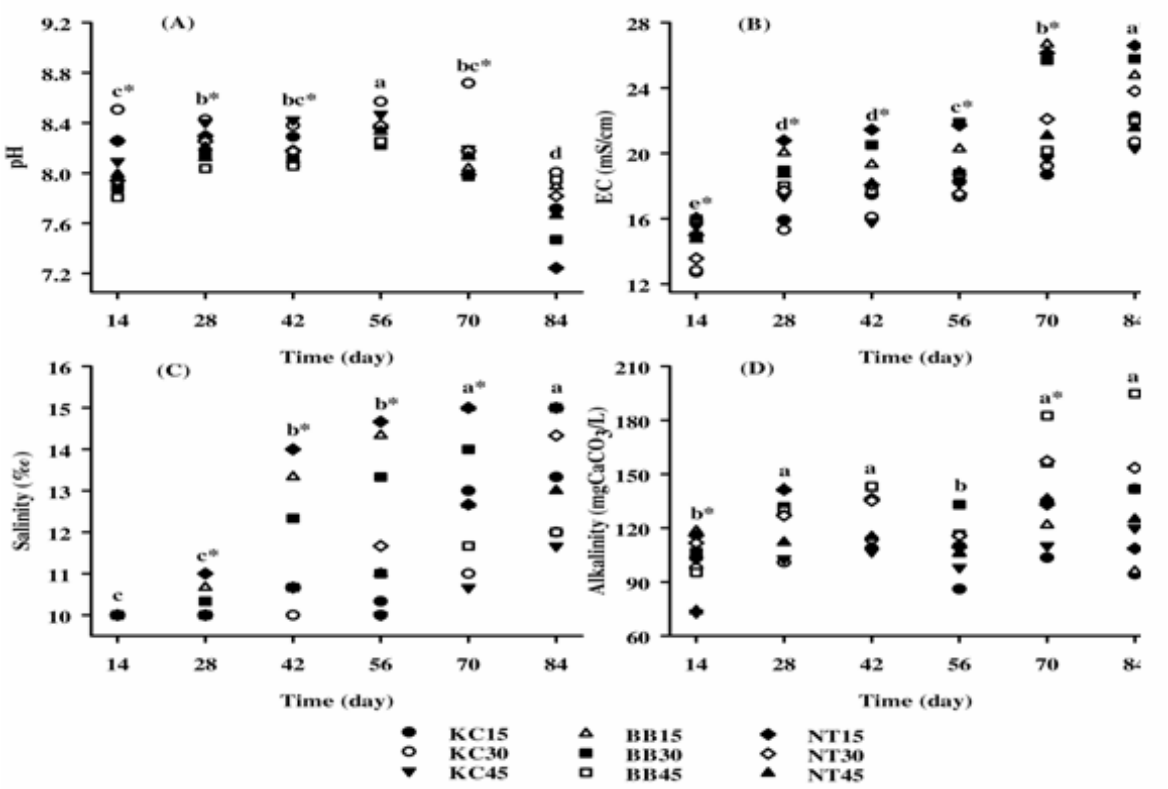

Figure 5. Values of pH (A), electrical conductivity (B), salinity (C) and alkalinity (D) in the water over time of *T. orientalis* (BB), *S. littoralis* (NT) and unplanted (KC) at +15, +30 and +45 cm water levels.

Notes: Values are the means of 3 replicates. Asterisk (*) indicates significant difference between treatments within sampling time. Different letter [a,b,c] indicates significant difference between sampling times based on a Tukey HSD test ($p<0.05$).

Electrical conductivity (EC) did not directly affect shrimp growth but it might cause effect on plant growth because EC was strongly related to salinity. The water EC had the same trend of salinity and reached the highest values at the end of the study ($p<0.05$; Figure 5B). The salinity stress inhibited plant growth and had the same symptom with water-stress. Water salinity level in this study was controlled at 10‰ in the added water during the study; however, the concentrations of salinity in all treatments tended to increase over 10‰ and reached to 15‰ at the end of the study (Figure 5C). It can be explained that at the sampling point salinity in the water

increased due to water loss via evapotranspiration and salinity even accumulated more at the end of the study ($p<0.05$; Figure 5C). However, they were in the permitted limit (5.0-35.0‰) for requirement on shrimp rearing water quality [12]. Konisky and Burdick (2004) [15] documented that only 50% of narrowleaf cattail (*Typha angustifolia*) transplants grown at the salinity of 10‰ in the field experiment were survived. Therefore, waterlogged and low nutrient concentration in water and sediment was not only the cause of growth and survival rate reduction in *Typha orientalis* [10], but salinity also might be the cause. The average of alkalinity in all the treatments was in the range of 101.8-143.7 $mgCaCO_3/L$ ($p<0.05$; Figure. 5D) which were in the permitted limit (60.0-180 $mgCaCO_3/L$) for requirement on shrimp rearing water quality [12].

3.2.3. Nitrogen and phosphorus concentrations in the water

Nitrite nitrogen (NO_2-N) is toxic to shrimp and exposure to high concentrations may cause retarded growth and mortalities [16]. The average NO_2-N concentrations in the treatments tended to reduce over time and increase again at the last sampling time ($p<0.05$; Figure 6A). Similar trend was observed in nitrate nitrogen (NO_4-N), ammonium nitrogen (NH_4-N) and total kjeldahl nitrogen (TKN) concentrations ($p<0.05$; Figure 6B, 6C & 6D) that reflected debris of plants and microorganism body degraded causing water pollution again. The treatments with +45 cm water level had the highest average NO_2-N concentrations. In general, water NO_2-N concentrations in the treatments were in the range of 0.03-0.3 mg/L, which was in the permitted range for normal shrimp growth [14]. However, NO_2-N concentration the in the unplanted treatments was slightly higher which was the same finding reported by Doan *et al.* (2016) [7] who using constructed wetlands planted *Typha orientalis* to purify intensive whiteleg shrimp wastewater. Gross *et al.* (2004) [16] suggested a safe concentration for whiteleg shrimp production in the ponds to be less than 0.45 mg/L NO_2-N.

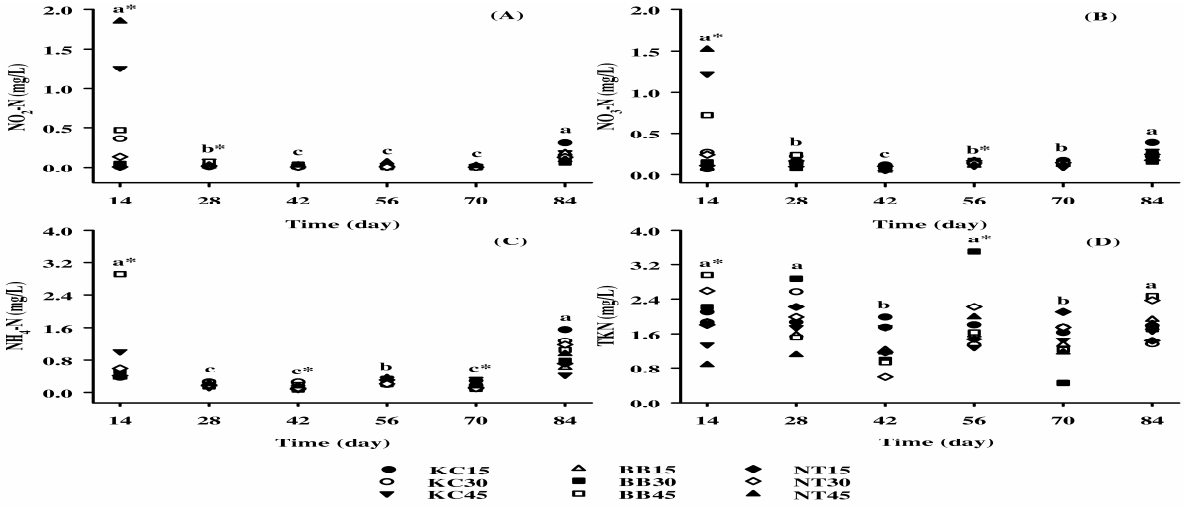

Figure 6. Nitrite nitrogen (A), nitrate nitrogen (B), ammonum nitrogen (C) and total kjeldahl nitrogen (D) in the water over time of *T. orientalis* (BB), *S. littoralis* (NT) and unplanted (KC) at +15, +30 and +45 cm water levels

Notes: Values are the means of 3 replicates. Asterisk (*) indicates significant difference between treatments within sampling time. Different letter [a,b,c] indicates significant difference between sampling times based on a Tukey HSD test ($p<0.05$).

The NO_3-N concentration is not harmful for shrimp growth, however, higher accumulation of NO_3-N might be toxic to shrimp [17]. In this study, NO_3-N concentration likely reduced overtime, except for the last sampling ($p<0.05$; Figure 6B). The NO_3-N concentrations in the treatments were negligible ranging from 0.14 to 0.37 mg/L. The NH_4-N concentrations in the treatments were in the range of 0.3-0.8 mg/L and the planted treatments had lower NH_4-N concentrations compared to the unplanted treatments ($p<0.05$; Figure 6C). The planted treatments with +15 and +30 cm water levels had lower NH_4-N concentrations because the plants grew well in these treatments resulting in a better NH_4-N uptake rate by the plants. This led to the lower TKN concentrations in those treatments too. However, TKN concentrations across treatments highly fluctuated and increased at the end of the study ($p<0.05$; Figure 6D) that might be due to algae and the death plant tissues returned nitrogen back into the water environment.

The concentrations of orthophosphate (PO_4-P) and total phosphorus (TP) of all the treatments had the same trend of reduction over time but increased at the last sampling point ($p<0.05$; Figure 7A & 7B). There were significant differences between the treatments within sampling time. Although P is not harmful to aquatic animals, P supports algae and aquatic plants growth. The increase in water level from +15 to +45 cm P concentrations increased. Rubio *et al.* (1997) [18] found that waterlogged soils increased the ability of plants to uptake phosphorus and could increase the soil phosphorus availability. However, repeated flooding can result in a phosphorus release from soils, which introduces additional phosphorus to soils and waterways [19, 20].

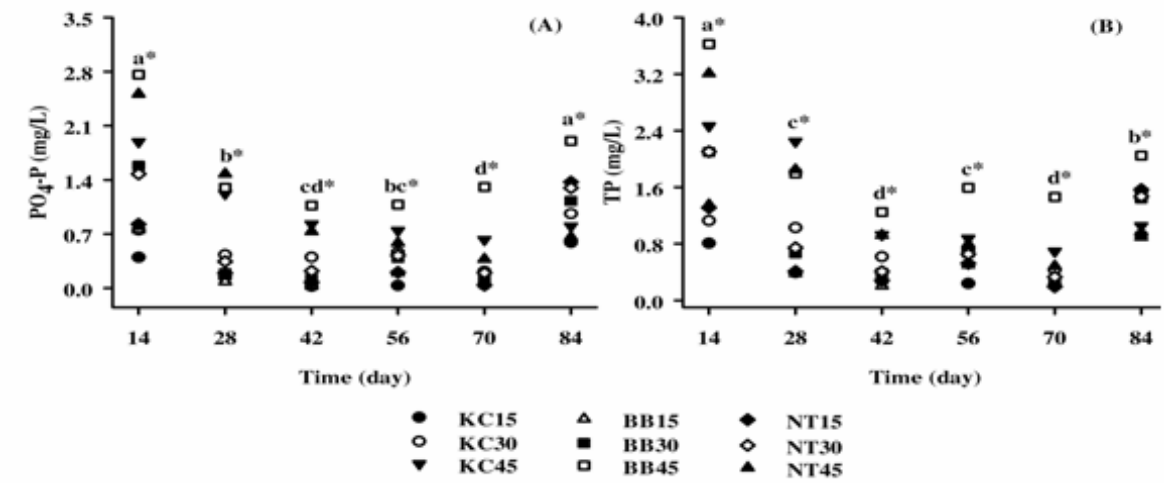

Figure 7. Orthophosphate (A) and total phosphorus (B) in the water over time of *T. orientalis* (BB), *S. littoralis* (NT) and unplanted (KC) at +15, +30 and +45 cm water levels

Notes: Values are the means of 3 replicates. Asterisk (*) indicates significant difference between treatments within sampling time. Different letter [a,b,c] indicates significant difference between sampling times based on a Tukey HSD test ($p<0.05$).

In sum, the concentrations of the tested water quality parameters across the treatments meet the Vietnamese standards requirement on shrimp rearing water quality. The treatments with plants helped to maintain better quality than that of the unplanted treatments. Water quality was relevant to plant growth response to the water levels. *Typha orientalis* grew better in the water levels of +15 and +30 cm that led to the lower

concentrations of nitrogen and phosphorus in the water.

4. CONCLUSION

In conclusion, the growth and biomass production responses of *T. orientalis* and *S. littoralis* was different at the water levels ranging from +15 to +45 cm. *Scirpus littoralis* grew well with lower survival rate and had no statistical reduction of biomass compared to *T. orientalis* at the highest water level of +45 cm while *T. orientalis* had the best perfromance in growth and biomass at water levels of +15 cm and +30 cm. The concentrations of the tested water quality parameters across the treatments meet the Vietnamese standards requirement on shrimp rearing water quality that can be reused for shrimp ponds. The treatments with plants helped to maintain better quality than that of the unplanted treatments. The results indicated that *S. littoralis* was the best of choice to grow in waterlogged condition of the shrimp pond for maintaining water quality. However, further studies must be conducted using a commercial shrimp pond or a settlement pond to grow *S. littoralis* together and/or separate to further assess the feasibility of using this species.

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AGRO-ECOLOGICAL ZONING AND LAND ADAPTATION ASSESSMENT FOR SUSTAINABLE FARMING IN THE BUFFER ZONE OF TA DUNG NATIONAL PARK

Nguyen Van Hiep[1,*], Luong Van Viet[1], Vo Dinh Long[1], Le Huy Ba[2]

ABSTRACT

Based on the criteria of agricultural ecological partitions, the research used agricultural ecological partition methods in the buffer zones of Ta Dung National Park. Besides, combine the GIS tool (Geographic Information System) to digitize, update information, overlap, zoning, build ecological partition maps, and adapt land units and partitions. Natural adaptive evaluation results and economic partitions for land adaptation for economic efficiency models are combined. Research results show that the buffer zone of Ta Dung National Park is divided into three different agricultural ecological areas including afforestation, perennial industrial crop, and fruit trees. In addition, from agricultural ecological partitions, three promising agricultural farming models are coffee, macadamia, and durian fruit specialized farming models. The research provides scientific data sources for conservation management models for other reserves based on the local community.

Keywords: *Ecological zoning, land, cultivation, National Park, Ta Dung.*

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1. INTRODUCTION

Ta Dung National Park in Dak Nong province is strategically located in economic, environmental, and national security position. Ta Dung National Park plays an important role in watershed protection, upstream of the two large rivers, Krong No - Serepok and Dong Nai river, which protects and maintains the ecological environment balance for the entire region [1], [2]. However, Ta Dung National Park is currently under significant pressure from the buffer zone's population through invasion activities, wood exploitation, and forest products, as well as hunting wildlife...[3], [4]. The effects of livelihoods on forest and environmental resources in the buffer zone have been pressuring the management of Ta Dung National Park [5], [6].

Therefore, the agricultural ecological partition and land adaptation evaluation in the buffer zone of Ta Dung National Park is necessary to establish a scientific foundation for applying conservation management models to other conservation areas based on the local community [7]. Research results can be applied to implement agriculture, protection of natural resources, and environment to ensure sustainable livelihoods for the population of the buffer zone in general and Ta Dung National Park in particular.

2. RESEARCH METHODS

- Information collection method: collection and synthesis of information on natural conditions, socio-economic, industry, and labor from annual reports of province, district, and commune levels (Dak Som commune of Dak G'long district in Dak Nong province, two communes of Phi Lieng and Da K'nang of Dam Rong district in Lam Dong province, the data from the Ta Dung National Park management board, the report of transfer of Ta Dung National Park in 2018).

- Interview survey method: investigate three buffer zone communes, including Phi Lieng, Đa K'Nang, and Dak Som with 120

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households/commune on livelihood resources [3] and the agricultural farming situation of the community in the research field [4]. Results of surveys on livelihood resources and factors influencing the agricultural farming model of the buffer zone of Ta Dung National Park have been presented in the research results of the authors according to the reference [3], [4].

- Geographic information system (GIS) and remote sensing (RS) method: this method is used to build land adaptation in five steps, which are as follows:

Step 1: Gather information about land use and natural conditions in the research area.

Step 2: Choose a background map, shape the image, and digitize it.

Step 3: Use GIS software to build monomer layers of soil, steep slope, irrigation, and the mechanical composition of the soil.

Step 4: Create a map of the land unit (LMU) using single layers.

Step 5: Develop land adaptation maps based on analytical tools in GIS software. From the land use requirements of the types of land use, based on the quality of the land of LMU, comparison to identify the appropriate types of the land of LMU.

- Agricultural ecological partitioning method: agricultural ecological partitioning plays a critical role in natural geography and environmental space, determining the specific ecological laws of each region and sub-region. Since then, the agricultural ecological map has been digitized, overlapped, and partitioned. Each ecological region has different soil and water properties, as well as different types of farming.

- Assessing land adaptation method: researching land properties and creating a land unit map. Investigate and assess the current state of land use, then select the land use type (LUT) for evaluation and determination of land use requirements. Decentralization (AHP) evaluates the land units' suitability for the selected land-use types. Connect via GIS through IDRISI software, MapInfo to display the distribution of evaluation results following adaptive partitioning.

3. RESEARCH RESULTS

3.1. Agricultural ecological partition of the buffer zone of Ta Dung National Park

The research area is comprised of six major soil types, with the red-yellow soil group accounting for the majority of the buffer area. The distribution of this soil group is as follows: the largest area is 17,353.77 ha of yellow-red soil on clay rock (Fs) (accounting for 65.5 percent), followed by red-yellow soil on magmatic acid rock (Fa) with an area of 4,438.94 ha (accounting for 16.8 percent), and the rest are yellow- brown soil on base and neutral magma (Fu) with an area of 1,153.28 ha (accounting for 4.4 percent) and yellow-brown soil on magmatic base and neutral rock (Fq) with an area of 1,342.79 ha (accounting for 5.1 percent).

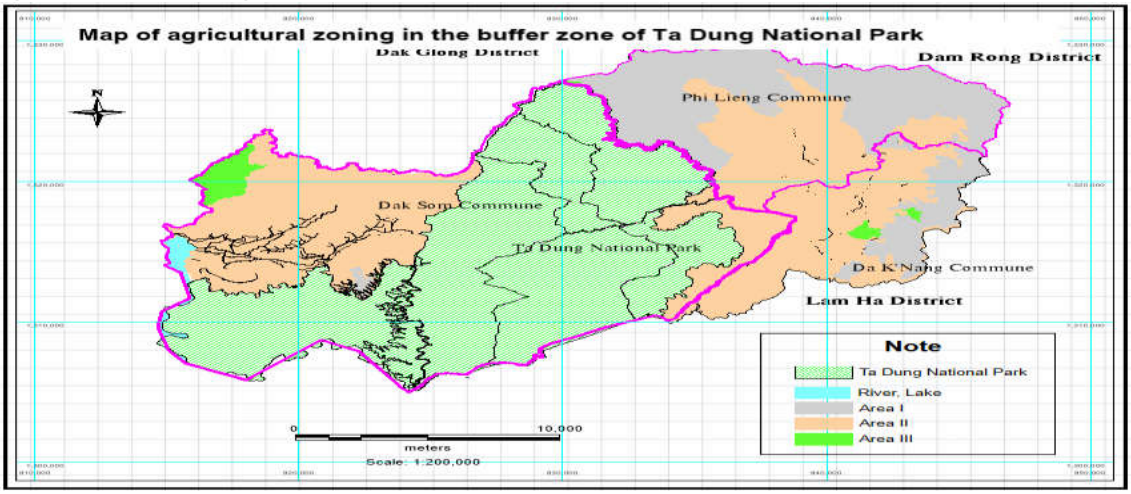

Figure 1. Map of agricultural zoning in the buffer zone of Ta Dung National Park

Red-yellow humus group in the mountain (distributed at belt-high ≥ 900 – 1,800 m) presents with red-yellow humus on clay rock (Hs) with an area of 1,407.23 ha (accounting for 5.3 percent). The valley soil group has an area of 314,43 ha due to the sloping product (D) accounting for 3.0 percent.

The natural land area in three buffer areas is 47,754.24 ha, accounting for 46.34% of the total buffer area. Agricultural farming is the main economic sector in the communes of the buffer zone of Ta Dung National Park. The production of industrial crops is considered a solution to poverty reduction for people living in the National Park buffer zone with agriculture revenue (mainly from coffee harvesting, high-yielding cassava, hybrid corn, and beans) accounting for a large proportion, estimated at over 70% of the total income.

In particular, the main crops in the three communes of Phi Lieng, Da K'Nang, and Dak Som are coffee with 6,449 ha, accounting for 84.4% of the agricultural farming structure.

Based on the terrain as well as analyzing the soil characteristics and the current status of agricultural land use, the buffer zone of Ta Dung is divided into three different agricultural ecological areas (Figure 1), including afforestation (Region 1), perennial industrial tree (Region 2) and fruit crops (Region 3).

- Region I: The afforestation area covering 8,301.25 ha, accounting for 17.38% of the area of three communes, including the entire high hills and mountains in the northeast of Phi Lieng and Da K'Nang commune. This region has a height of 900-1,400 m, soil is mostly red-yellow on clay rock (Fs) and red-yellow soil on magmatic acid rocks (Fa). In general, with current natural conditions, it is suitable for planting forestry, large wood, and impurities.

- Region II: The ecological perennial plant area with the most area of 11,141.05 ha, accounting for 37.50%, the outstanding feature of this region is the average higher from 750 m to 1,000 m, can specialize in coffee trees. This area has the advantage of favorable irrigation systems and soil is mainly red-yellow on clay and yellow-brown soil on magmatic base and neutral rock (Fq), suitable for coffee tree development.

- Region III: Ecological area of fruit trees and other annual crops, with the smallest area of 272.40 ha, accounting for 0.57% of three communes. The characteristic of this region has the lowest terrain from 580 m to 900 m, the highest in Da K'Nang commune with 1,048 m. The soil in this region mainly red -yellow soil on clay rock (Fs) is suitable for many crops, but it is necessary to supplement the nutrients needed to meet the nutrition of plants. Some ideal plants for planting such as ginger, potato, sweet potato, cassava, tuber, or fruits such as jackfruit, guava, lemon, pepper, plum, and banana,...

3.2. Assessment of land suitability for sustainable farming in the buffer zone of Ta Dung National Park

Based on the socio-economic planning of the region, the results of the soil research survey, and the actual survey results on the land use situation of the buffer zone of Ta Dung National Park, the factors are considered to build a map of the land unit, as a basis for assessing the ability to adapt the land, including the characteristics of the mechanical components, exposed stone head, the slope, the soil, the irrigation conditions to create a land unit map. The results showed that the research area had all 28 land units.

Table 1. Statistic of land plots

Land unit (LMU)	Area (ha)	Land unit (LMU)	Area (ha)
1	630.92	15	0.10
2	155.15	16	75.12
3	290.03	17	369.61
4	1,941.24	18	67.66
5	2,207.67	19	606.79

6	158.91	20	439.53
7	97.02	21	640.65
8	264.44	22	389.38
9	32.76	23	6,266.27
10	342.71	24	2,516.50
11	197.78	25	6,057.39
12	89.38	26	1,153.28
13	84.14	27	15.72
14	0.43	28	1,332.25

(+) Valley land group (D): Valley land has an area of 786.07 ha and is represented by the symbols TD6 and TD13 (Phi Lieng commune). This type of soil does not promote much growth of the tree. In terms of slope, this is level II (> 3^0-8^0) and does not interfere with agricultural farming, but it is a low-lying area that frequently floods during the rainy season. The properties have been taken on average, the two models are mechanical C (light), hydrolysis and high acidity (respectively 10.15-8.15 meq/100 g soil, content pH_{KCl} <4.5 (3.66); the soil is too acidic leading to a minimum of plant growth. The mechanical composition includes 35.9% of clay, 52.35% of limonite, and 11.75% of sand; CEC 7.75 meq/100 g soil. Content nutrition includes 0.23% N, 0.12% P_2O_5, 0.78% K_2O. The exchange content of Ca^{++}, Mg^{++}, and K^+ in the soil are low, plants may be lacking an equivalent value of 0.35-0.15-0.57 meq/100 g soil.

Valley land is distributed in Phi Lieng and Da K'Nang communes, and this group is now being planted with coffee trees and large timber trees in Phi Lieng and Da K'Nang communes. People in this area are limited to planting on the valley land and a small area of coffee planting.

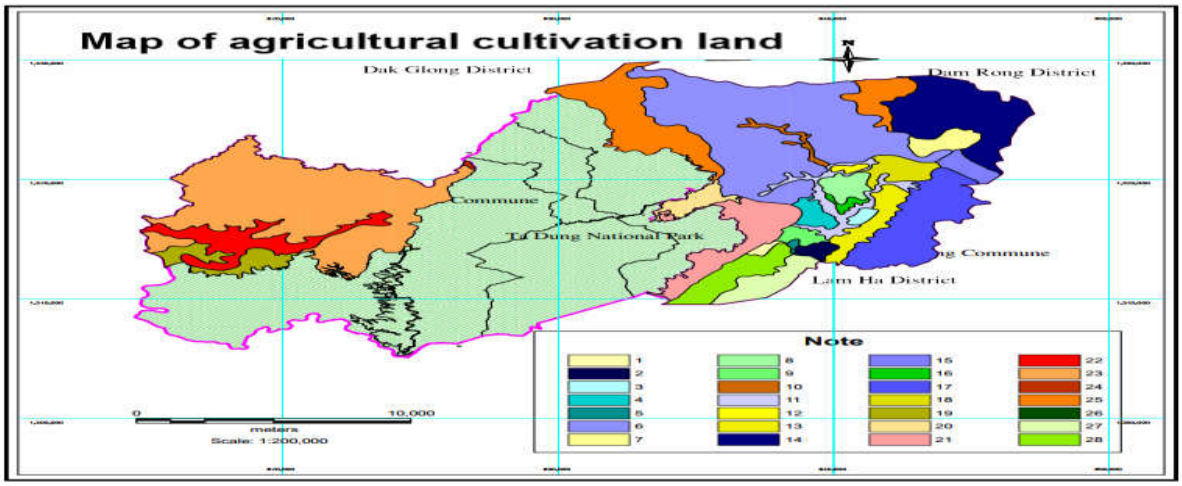

Figure 2. Map of agricultural cultivation land (LMU)

(+) Yellow-brown soil type on magmatic base and neutral rock (Fq): this soil type has an area of 1,153.28 ha; Fq representative sample symbolizes MC1 (main form) and TD1 (only available in Dak Som commune). This soil type represents full good properties such as reddish-brown soil, moister property, and more moist climates which can grow many crops. Regarding the slope here, level I (<3^0) does not interfere with agricultural farming. Mechanical cultivation layer with D (average) and low hydrolysis and exchange acidity content (average values with 5.31 meq/100 g soil and 0.38 meq/100 g soil, respectively; the content of pH_{KCl} 4.4-6.21 (Light acidic layer of A). The

mechanical composition includes 26.66% of clay, 15.47% of limonite, 49.15% of sand; CEC 8.15 meq/100 g soil. Content nutrition includes 0.26% N, 0.08% P_2O and 0.14% K_2O). Exchange cations Ca^{++}, Mg^{++}, and K^{+} are abundant in soil with values of 3.90-0.30-0.14 meq/100 g soil, respectively.

Yellow-brown soil on magmatic base and neutral rock (Fq) is only distributed in Dak Som commune; now this soil type is being planted with coffee trees for high productivity.

(+) Red- yellow soil on clay rock (Fs): This soil type covers an area of 17,353.77 ha which is the largest soil type in the three studied communes. Representative soil samples with Fs sample symbols: MC2 (main sample) and TD2, TD3, TD4, TD7, TD8, TD9, TD12, TD14. This soil is generated from sandstone shale, clay schist, mica schist, gneiss,... Mechanical composition is medium and heavy with exposed rock structure, and rather porous topsoil. Humus content is good, total nitrogen is average (0.25% N), total phosphorus 0.07% P_2O_5, and total potassium 0.02% K_2O, as well as easily digestible as Ca^{++} (0.83 meq/100 g soil), Mg^{++} (0.24 meq/100 g soil), K^{+} (0.09 meq/100 g soil) are poor.

The slopes here are of grades I and II (< 8◦) which do not cause much impediment to agricultural cultivation. The content of hydrolytic acidity and exchange acidity (average of the main sample's cultivation layer is 9.41-1.24 meq/100 g soil, respectively), pH_{KCl} content of 4.06 (<4.5 is acidic). The mechanical composition includes 40.93% of clay, 14.24% of limonite, and 42.34% of sand; CEC 7.06 meq/100 g soil. Currently, this soil type is being planted with many types of trees such as coffee, big trees, banana, and macadamia trees bringing high economic value.

(+) Red-yellow soil on magmatic acid rock (Fa): this soil type has an area of 4,438.94 ha, located entirely to the east, bordering Lam Ha district, belonging to Phi Lieng and Da K'Nang communes. Representative soil samples Fa with sample symbols: MC3, MC4 (main sample), and TD10. This soil type has light mechanical composition, often poor structure, and a thin soil layer (usually less than 1.2 m). This soil is acidic (pH_{KCl} - 3.94), poor in humus, 0.14% N, 0.06% P_2O_5, and 0.89% K_2O. The potassium content is better than the yellow-red soil on clay and metamorphic rocks, the soil has low clay content (28.77%) so the absorption capacity is low, and the ability to retain water and nutrients is poor.

This soil type is being used to grow large trees, mixed crops, intercropping coffee - macadamia, banana.

(+) Yellow-brown soil on base and neutral magma (Fu): this soil type has an area of 1,342.79 ha, distributed mainly in the Da K'Nang commune. Representative soil samples with Fq sample symbols: MC5 (main sample) and TD11. The soil has heavy mechanical composition, the thick soil layer is over 2 - 3 m, the soil is acidic (pH_{KCl} - 4.06-4.11), and the total nitrogen and phosphorus are quite good (0.17% N and 0.05% P_2O_5, respectively). Total potassium is poor (0.03% K_2O), and easily digestible substances are quite good (Ca^{++}, Mg^{++}, and K^{+} values are 0.69-0.07-0.11 meq/100 g soil, respectively); average absorption capacity (7.6-5.5 meq/100 g soil).

This soil type is suitable for many perennial crops such as existing coffee but needs to be supplemented with organic matter, fertilizer, and erosion control.

(+) Red-yellow humus on clay rock (Hs): This soil type covers an area of 1,407.23 ha, mainly distributed in Da K'Nang commune, bordering Ta Dung National Park. Representative soil sample with Hs sample symbol: TD5. This soil has a medium and light texture. Soil response from acidic to very acidic pH_{KCl} - 3.61. Phosphorus nutrient content 0.86% P_2O_5, but other nutrients such as total nitrogen and potassium as well as digestibility are poor (0.278% N and 0.16% K_2O, respectively). This soil is currently being used effectively in coffee production.

Table 2. Description of land units

Ord.	Soil	Ground compartment	Characteristics
1	Valley soil as a result of steep product accumulation (D)	11, 14	Advantages: The valley soil group has almost no advantages, only advantages when improving the land. Disadvantages: low yield, low terrain frequently flooded → gley occurs, acidic reaction, many toxins affect plants, fast drainage does not accumulate for plants.
2	Red-yellow soil on magmatic acid rock (Fa)	18, 19, 27	Advantages: potassium content is better than yellow-red soil on clay and metamorphic rocks. Disadvantages: this soil type has a light mechanical composition, frequently poor texture, and strong erosion, so its ability to retain water and nutrients is poor.
3	Yellow-brown soil on magmatic base and neutral rock (Fq)	1, 2, 3, 4, 6, 9, 10, 12, 13, 16	Advantages: almost none Disadvantages: light mechanical composition, very high sand grain rate, unstructured soil, or very poor texture. Soil is eroded, and leaching occurs strongly.
4	Red-yellow soil on clay rock (Fs)	5, 7, 8, 15, 17, 20, 21, 22, 25, 28	Advantages: medium and heavy mechanical composition, the texture of lumpy and granular, and the topsoil is quite porous. Disadvantages: other nutrients such as total phosphorus and potassium are poor as well as easily digestible.
5	Yellow-brown soil on base and neutral magma (Fu)	24	Advantages: this soil is used for agricultural production with many perennial or annual dry crops. Plants were grown on Fu soil grow well and give good yields. Disadvantages: a thin effective soil layer due to many shallow formations, limiting the growth of some perennial plants with deep roots such as durian.
6	Red-yellow humus on clay rocks (Hs)	23, 26	Advantages: this soil has a good content of humus and nitrogen in the surface layer and rich exchangeable potassium. Disadvantages: medium-light mechanical composition, easy to erode, Ca^{2+}, Mg^{2+} are washed away strongly, the soil has an acidic reaction

3.3. Elaboration of land use requirements and decentralization for LUT's current and prospective

Based on the results of analysis and assessment of the current state of land use, natural socio-economic conditions, and the market in the study area, the results have presented three types of promising LUTs: coffee tree LUTs, macadamia tree LUTs, and specializes in durian cultivation. The research used FAO's greatest limiting factor assessment method. Specifically, the suitability of a land unit to a type of land use is the most appropriate classification of the characteristics of the land. In other words, as long as one of the natural conditions (soil, mechanical composition, slope, exposed rock, and irrigation conditions) is not favorable, a certain type of land use will not be possible although the remaining natural conditions are favorable. Restrictions are often represented as land rank, for each feature or property, a class S1 (very suitable), a class S2 (moderately suitable), a class S3 (poorly suitable), and a class N (not suitable and cannot be adjusted) can be defined.

The average adapted area for coffee in the study area is 8,087.48 ha, poorly adapted is 16,201.31 ha, and non-adapted is 2,134.03 ha, there is no highly adapted area. Respectively, suitable area of macadamia tree: (S1) 6,564.58 ha, (S2) 18,782.14 ha, (S3) 786.07 ha, no non-adapted area. The adaptive area of durian is: (S1) 6,564.58 ha, (S2) 17,724.21 ha, (S3) 1,347.96 ha and non-adapted 7,860.69 ha.

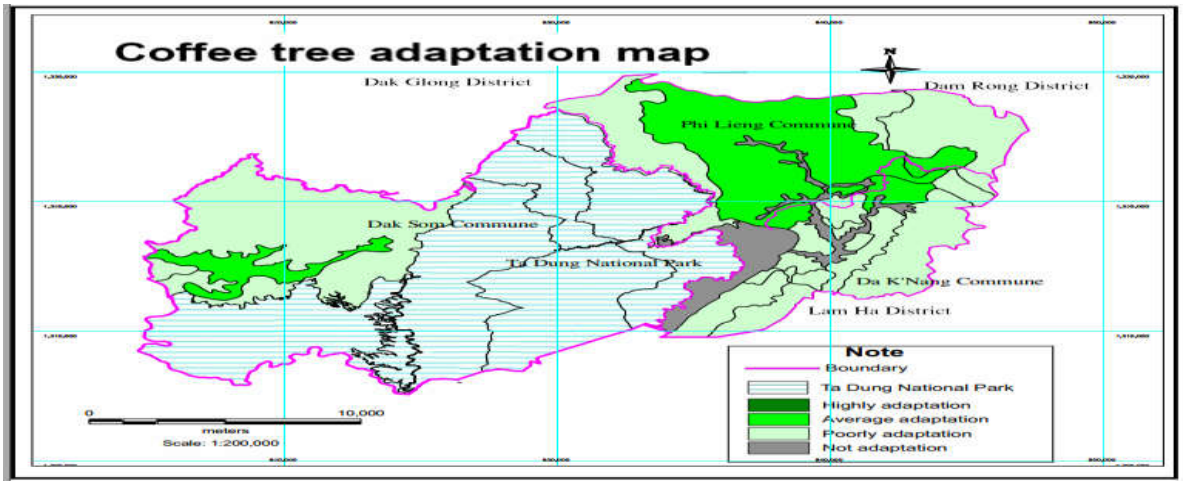

Figure 3. Coffee tree adaptation map

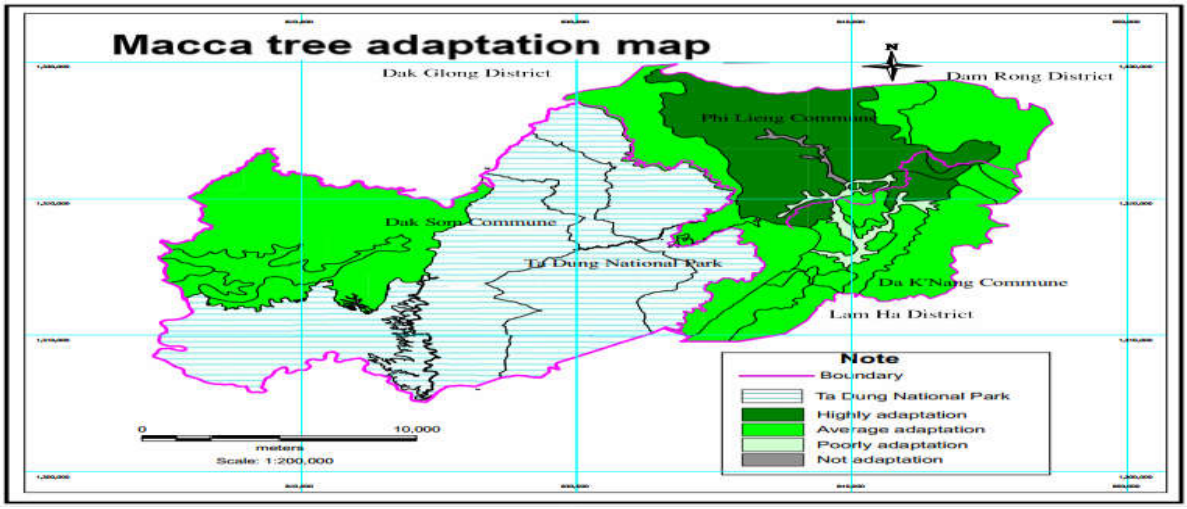

Figure 4. Macca tree adaptation map

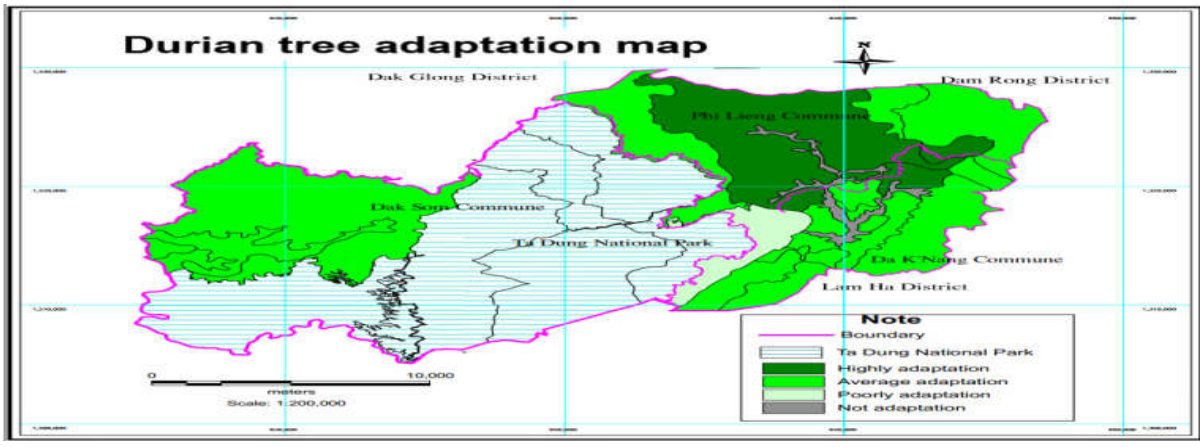

Figure 5. Durian tree adaptation map

The limiting factors of the above farming models in the study area are the inhospitable nature of the valley soil, the majority of mechanical components having a light structure, and the content of soluble nutrients that are easily washed out. drifting on steep slopes of red-yellow soil on acidic igneous rocks. In addition, there are exposed rocks in some places near Ta Dung National Park and semi-active irrigation conditions in locations far from water sources.

4. CONCLUSION

The study assessed the appropriate level of agricultural farming models in the buffer zone of Ta Dung National Park to propose sustainable farming models and minimize the impact on forest resources. Three different agricultural ecological areas were divided including afforestation (Region 1) that is suitable for planting forestry, large tree; perennial industrial crop that is specialization for coffee (Region 2) and the ecological area of fruit trees such as ginger, potato, sweet potato, cassava, or fruits such as jackfruit, guava, lemon, plum, pepper, banana, ... (Region 3).

The results of adapting the current situation of crops (coffee) are very suitable for natural conditions in the three communes of Dak Som, Phi Lieng, and Da K'nang. Three promising agricultural farming models were coffee, macadamia, and durian fruit planting model, which resulted in the map for assessing the adaptability of the two models based on ecological requirements.

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ON THE PROBLEMS AND COUNTERMEASURES OF AGRICULTURAL TRADE BETWEEN GUANGXI AND ASEAN UNDER THE BACKGROUND OF EPIDEMIC SITUATION

Mai X. Bui[1,*], Yu Zh. Lu[2]

ABSTRACT

To prevent the spread of Covid -19, China and Asean countries have introduced a series of strict pandemic prevention policies. Although these policies have prevented the spread of Covid - 19, but they have had a great impact on agricultural trade between China and Asean countries. In this paper, the author synthesizes the Covid-19 prevention and control policies of China and Asean countries, analyzes the changes of Guangxi's import and export of that epidemic on the markets between Guangxi and Asean, and the types of agricultural products. Finally, the author makes some recommendations for Guangxi province's import and export activities. China's Guangxi Province needs to strengthen trade negotiations with Asean countries to stabilize agricultural products' import and export, strengthen its ability to resist risks. Guangxi actively integrates into The Guangdong-Hong Kong-Macau Greater Bay Area, improves the structure of the product industry chain, improves brand awareness, enhances the initiative in trade activities.

Keywords: *Covid -19, China - Asean free trade area, agricultural trade.*

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1. INTRODUCTION

At the end of 2019, Covid - 19 broke out in Wuhan-China, after which it spread all over the world. On November 3, 2019, the World Health Organization (WHO) announced that Covid - 19 can be characterized as a pandemic [1], officially recognizes Covid - 19 has entered a global pandemic. Covid - 19 has spread from a single public health event to a food security crisis. It shows the remarkable features of comprehensiveness and profoundness, its impact on the global economy, politics, society and trade is all-encompassing. Covid 19 has intensified the downside risks of the global economy, and the instability and uncertainties have increased significantly [1]. The pandemic has caused a serious impact on the world economy by suppressing demand and interrupting supply [2], and the agricultural product industry has received the most serious impact. The impact of the pandamic on agricultural trade between China and Southeast Asian countries can be discussed in two stages: The first stage is the early stage of the pandemic: in this time, demand in the Chinese market has declined and imports have decreased. As a result, China's agricultural imports from Asean countries have been greatly reduced. The second stage is the spread of the pandemic: The pandemic has spread rapidly in Southeast Asia, has brought an external effect to trade activities and supply chain in Asean [2]. Since 13 January 2020, all Asean member states have reported confirmed cases of Covid - 19, and the number of cases has increased dramatically in a short period of time, with the pandemic becoming increasingly

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serious. Due to rapid urbanization and consumption upgrades, Asean countries faced long-term challenges to food security before the pandemic [2], Covid - 19 has impacted the sustainable and stable supply of the Asean food system. The severe pandemic situation has led Asean member states to adopt strict pandemic prevention and control measures on entry and exit activities, resulting in certain restrictions on import and export trade, and agricultural trade is no exception. Asean, as China's largest trading partner in goods, is also the largest trading partner of Guangxi and the largest market of Guangxi's agricultural products trade. Its agricultural products trade is restricted, which will have a certain impact on the import and export of Guangxi's agricultural products and bring hidden dangers to regional food security. Therefore, in the context of the strict pandemic prevention and control measures taken by Guangxi and Asean countries, combined with the policies and measures taken by Asean countries to deal with the pandemic, the future development direction of agricultural trade in Guangxi under the influence of the pandemic is discussed. As for the cooperation between Guangxi Asean even China-Asean to jointly fight the pandemic, maintain the stability of regional agricultural market and ensure food security, all of them have very important practical significance.

2. THE IMPACT OF COVID - 19 ON GUANGXI'S IMPORT AND EXPORT OF ASEAN AGRICULTURAL PRODUCTS TRADE

2.1 The impact of the pandemic on the total trade volume of agricultural products between Guangxi and Asean

Since the establishment of China-Asean free trade area in 2010, trade between China and Asean countries has become increasingly close. Guangxi, benefiting from its geographical position and policy advantages, has developed rapidly in agricultural trade with Asean countries. According to Guangxi Statistical Yearbook 2020 [2]. In 2019, the total export of Guangxi to Asean countries was 233.465 billion yuan, an increase of 13.3% compared with 2018, accounting for 49.73% of the total export of the region. With the strength of occupying almost half of Guangxi's import and export market, Asean firmly sits on the top position in Guangxi's foreign trade. In agricultural trade, Guangxi's agricultural trade with Asean grew at an average annual rate of about 19 percent from 2002 to 2017, according to Chinese customs data. In 2018, the trade volume of agricultural products in Guangxi reached 6.9 billion US dollars, ranking ninth in China. Among them, the trade volume of agricultural products between Guangxi and Asean reached us $2.92 billion, up 21.2% year on year, ranking among the top five in China.

Table 1. Main response measures of Asean countries in the first half of the year

Commencement date	Countries	Main contents of measures
March 16	The Philippines	Lockdown measures were implemented and later extended to Luzon
March 16	Thailand	Ubon closed the Thai-Lao-Cambodia border free trade Zone
March 18	Malaysia	Enforcement action control order for a period of two weeks
March 18	Vietnam	Border ports between Vietnam and Cambodia will be temporarily closed, and visas for foreigners entering Vietnam will be suspended
March 19	Brunei	Imposed a total travel ban and ban all locals and foreigners in Brunei from leaving the country

March 20	Indonesia	The issuance of visas to all countries is suspended
March 20	In Cambodia,	To temporarily close cambodia-Vietnam border ports
March 20	Brunei	The issuance of arrival visas and tourist visas will be suspended within 30 days
March 21	Myanmar	Stop processing arrival visas and electronic visas
March 22	The Philippines	The entry of foreigners is temporarily prohibited
March 23	Singapore	The Thai-Malaysia land pass and Thai-Cambodia border crossings will be temporarily closed
March 24	Vietnam	Vietnam's General Administration of Customs ordered a ban on all rice exports from March 24
March 26	Thailand	The imposition of a state of emergency, including the closure of entry and exit routes, crossings, border stations or temporary areas
March 30	Laos	A nationwide lockdown is in place until April 19
April 1	Vietnam	A 15-day quarantine was imposed, with the highest risk of outbreaks being extended until April 22 or 30
April 7	Singapore	Enhanced security quarantine measures for four weeks ending May 4
April 15	Laos	Extended the nationwide lockdown until May 3

Data source: Arranged based on research data of Zhejiang Institute of Standardization and other authors

Table 2. Restrictions on import and export trade by Asean countries in the first half of 20 years

Countries	Main contents of measures
Singapore	Stricter precautions will be put in place for ships coming from or visiting China, and cargo quarantine procedures will be increased
The Philippines	Cancel the right of ships from all affected ports to dock directly, and prohibit the crew of such ships from coming ashore
Thailand	We will close some border ports and strengthen quarantine on imported and exported agricultural products
Vietnam	Land port clearance will be closed. Containers imported from China will have to undergo 14 days of virus quarantine before they can be declared for import
Malaysia	At the port, all ships from China will be quarantined until they are inspected by Malaysian health ministry officials

Data source: Official website of each country and customs release information collation

However, due to the impact of the worsening Covid - 19 situation, the prevention and control measures of Asean countries are becoming increasingly strict. From March to April, when the pandemic was at its peak, Asean countries introduced a series of pandemic prevention and control measures to control the development of Covid - 19 in their countries. Among the pandemic prevention and control measures, Asean countries mainly restrict the flow of nationals through curfews and city closures, and prevent the inflow of migrants by closing border ports and suspending visa issuance (see Table 1 for details). The restrictions on agricultural trade are mainly reflected in strengthening the quarantine of imported products and increasing quarantine

measures. Especially for products and ships from China, the prevention and control measures are stricter and the procedures are more complicated (see Table 2 for details).

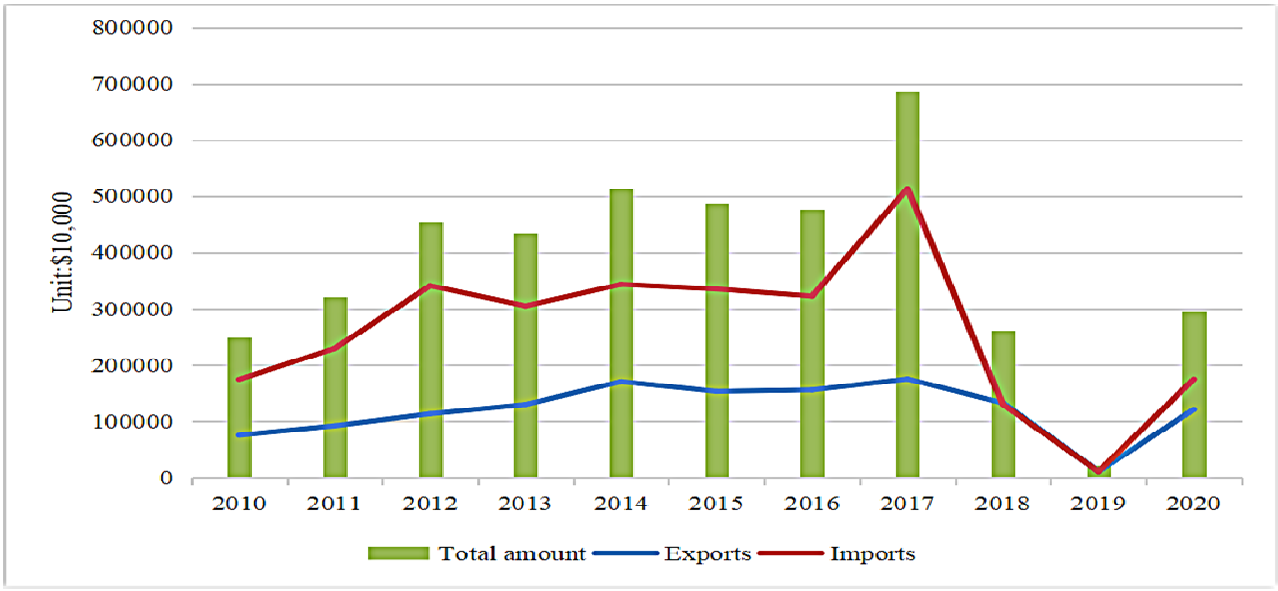

Figure. 1. Trade volume of agricultural products import and export between Guangxi and Asean in recent ten years

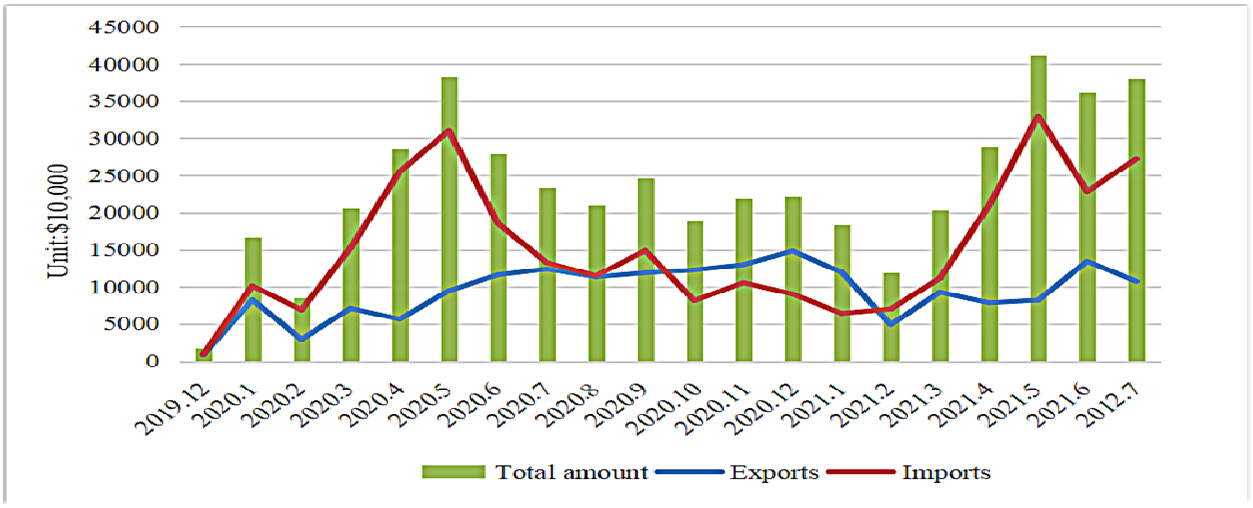

Figure 2. Import and export of agricultural products between Guangxi and Asean from December, 2019 to July, 2021

Data source: Nanning Customs [6]

Before looking for the trade data of agricultural products between Guangxi and Asean in 2020, we believe that the trade volume in 2020 will decline to some extent compared with that before. However, according to Figure 1, it is not difficult to find that the trade volume of agricultural products between Guangxi and Asean in 2020 will increase to some extent compared with that in 2018 and 2019. Moreover, the total trade in agricultural products increased by 12.7 percent in 2020 compared to 2018, the year with the closest trade volume. It is incredible that in the face of the spread of the pandemic, Guangxi's import and export of agricultural products to Asean has increased rather than retreated. From Figure 2, we can clearly see that the rapid growth of agricultural imports is the main reason for the rapid growth of agricultural trade volume in 2020,

and since February 2021, the import amount of Guangxi's agricultural products to Asean has increased more rapidly.

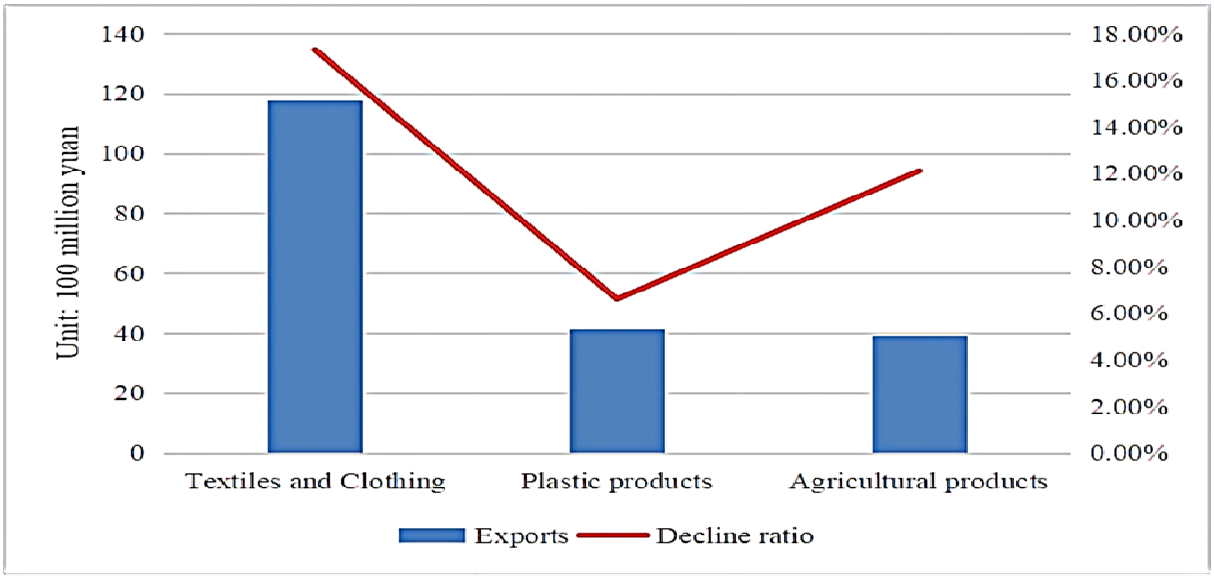

Figure 3. Guangxi exported Asean labor-intensive products in the first quarter of 2020

Data source: Nanning Customs [6]

According to Figure 2, it is not difficult to find that the trade volume of agricultural products between Guangxi and Asean was significantly affected in the first half of 2020 when the pandemic outbreak was the most serious, with the most serious decline in February 2020. This was also the peak of the pandemic in China. At the same time, Asean countries also began to gradually strengthen the degree of agricultural products entry restrictions. As Asean countries strengthen the degree of restrictions on agricultural products, resulting in Guangxi's agricultural exports to Asean weak. In the following March to June, due to the improvement of the pandemic situation in China, the market vacancy caused by the pandemic period led to a rapid increase in the import amount, so the total trade volume kept rising. Since the pandemic was gradually brought under control, that is, since the second half of 2020, the total trade volume has gradually stabilized at around us $2 billion.

At this point, the decline in the market demand for agricultural products caused by the pandemic and the lack of logistics and transportation of agricultural products have had a significant impact on the trade of agricultural products between Guangxi and Asean in the first half of 2020. According to the data released by Nanning Customs (see Figure 3 for details), only in terms of exports, in the first quarter of 2020, compared with other exports, the export of labor-intensive products, mainly agricultural products, dropped significantly. The export of labor-intensive products reached 21.24 billion yuan, down 25.7%, or 4.9 percentage points narrower than the previous four months. Among them, the export of textiles and clothing reached 11.77 billion yuan, down 17.3%. The export of plastic products was 4.16 billion yuan, down 6.6%. Agricultural exports totaled 3.94 billion yuan, down 12.1%. From Figure 1, we can clearly see that agricultural products are the most affected, second only to textiles and clothing.

2.2. Impacts of pandemic situation on market structure of agricultural products trade in Guangxi

In recent years, Asean has been Guangxi's largest trading partner, and its trade volume has been growing at an annual rate of almost 10

percent. In Asean, Vietnam, with the advantage of its geographical proximity to Guangxi, firmly occupies the top position in Guangxi's import and export market to Asean, followed by Indonesia, Thailand, Singapore and Malaysia. According to the statistics of Guangxi agricultural department, Guangxi's agricultural exports to Vietnam have occupied about half of Guangxi's agricultural exports for many years. Meanwhile, Guangxi is also Vietnam's largest fruit export market. At the same time, with the advantage of the geographical position of up to 9 land borders, a large number of commodities are continuously exported to The Asean region through ports of Guangxi and Vietnam in the form of small border trade, which is also the main trade form between China and Asean countries and regions.

Table 3. Total trade volume of agricultural products between Guangxi and the five Asean countries in the first half of 2020

Countries	January ($10,000)	The same period as the previous two years (%)	February ($10,000)	The same period as the previous two years (%)	March ($10,000)	The same period as the previous two years (%)
Vietnam	11642	- 9.80%	6891	- 31.90%	12812	- 21.80%
Thailand	5805	- 3.20%	1395	17.80%	7868	- 42.80%
Indonesia	444	1.20%	889	54.30%	724	- 57.90%
Malaysia	174	2.40%	330	- 73.20%	294	- 63.80%
Philippines	225	1.80%	154	20.30%	527	50.40%

April ($10,000)	The same period as the previous two years (%)	May ($10,000)	The same period as the previous two years (%)	June ($10,000)	The same period as the previous two years (%)
14515	- 11.80%	13286	- 3.20%	14569	- 1.80%
15140	32.80%	24831	123.70%	13784	- 14.70%
780	86.20%	1265	- 28.40%	876	52.60%
180	- 79.80%	24831	86.70%	368	80.30%
317	26.70%	276	17.40%	271	3.80%

Data source: Nanning Customs [6]

As the pandemic continues to worsen, China and Asean countries have taken measures to close ports and border ports, resulting in product accumulation, customs clearance delays, ship delays and aircraft grounding. The logistics and transportation of agricultural products are limited, and the demand for agricultural products is reduced due to the restrictive measures of domestic population flow in various countries. As a result, the total trade volume of agricultural products between Guangxi and Asean countries is hit to a certain extent (see Table 3 for details). As can be seen from Table 4, the impact of the pandemic on the trade of agricultural products between Guangxi and Asean countries is mainly reflected in the first three months, and the total trade volume of four of the five countries declined significantly. In mid - March, Guangxi's trade with

Malaysia and Indonesia dropped by more than 50 percent. In addition, Take Vietnam, Guangxi's largest trading partner with Asean, as an example. Due to the huge market of Vietnam and its importance in agricultural trade between Guangxi and Asean, Guangxi still receives agricultural products from Vietnam during the pandemic. On the Vietnamese side, since January 31, 2020, the Vietnamese government has signed relevant directives requiring the temporary closure of ports to discourage trade with China during the pandemic period [6]. It also took excessive control measures against China, including the suspension of flights and 14-day quarantine of imported cargo and ships. Due to the closure of border trade ports, a large number of agricultural products have been stranded at China-Vietnam border ports, mainly watermelon, mango, dragon fruit and other fruits and aquatic products exported from Vietnam to China. It was not until February 7th that the Vietnamese government allowed goods to pass through the border [7]. However, due to the early closure, overstocked goods and the slow speed of goods, the current efficiency of goods clearance is low [7]. The closure of ports and ports has dealt an obvious blow to small border trade, the main form of trade between Guangxi and Vietnam. It can be clearly seen from Table 4 that from February to May 2020, the import and export of small border trade (except trade among border residents) all declined, and February and March was the most affected by the decline of 25.5% and 12.8% respectively compared with the same period of last year. To sum up, due to the impact of the pandemic, the bilateral trade volume of agricultural products between Guangxi and Vietnam in the first three months dropped by about 20% year-on-year. Fortunately, since March, the prevention and control measures of various countries have been gradually improved and stricter, and the pandemic in China has gradually been effectively controlled, and the trade volume of agricultural products has gradually recovered and increased.

Table 4. Small border trade (except trade among border residents) from February to May, 2002

Border small trade (all goods included)	Total import and export	Export	Import	Total in the same period last year (± %)	Export (± %)	Import (± %)
February	8903.6	8333.398	570.201	25.5	25.7	22.4
march	11592.25	11196.946	395.303	12.8	12.8	13.1
April	9558.284	9098.72	495.565	1.64	2.83	29.9
May	1072.4835	10526.013	198.823	3.8	3.7	4.1

Data source: Nanning Customs[7]

2.3. Impacts of pandemic on trade structure of agricultural products between Guangxi and Asean

Since 2004, the tariff reduction policy has been gradually implemented between China and Asean countries, and then the establishment of China-Asean free trade area, a large number of products have been reduced or even exempted. The reduction of tariffs has greatly promoted the development of agricultural trade between China and Asean countries. From the perspective of trade structure, agricultural products exported by Guangxi to Asean are mainly aquatic seafood and vegetables, while imported agricultural products are mainly cassava, longan, dragon fruit and mango. Due to the impact of the pandemic, Guangxi's export of agricultural products to Asean has been hit hard, among which the three categories most affected are vegetables, aquatic

seafood and fruit. The export of these three categories is 1.14 billion yuan, 360 million yuan and 160 million yuan, down by 29%, 28.8% and 54.8%, respectively. The export of fruit has even dropped by more than 50%.

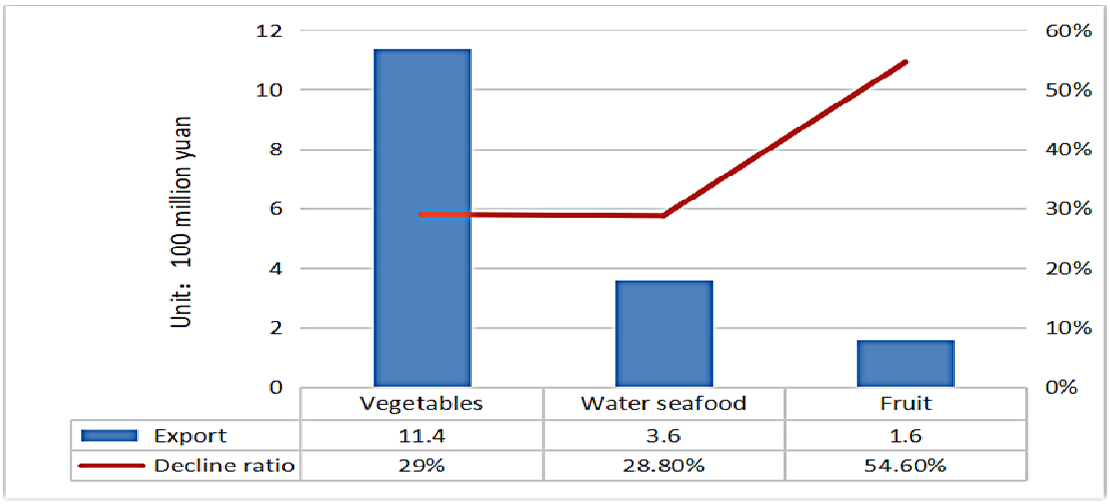

Figure 4. Mainly affected exports of agricultural products in the first quarter

Data source: Nanning Customs [6]

The comparison between the main agricultural products exported from Guangxi to Asean in May 2020 and the main agricultural products imported from Guangxi to Asean in May 2020 shown in Figure 5 and Figure 6 shows that the import of agricultural products from Guangxi to Asean shows a certain trend of growth while the main agricultural products exported from Guangxi to Asean show a trend of decline. The main export of agricultural products except tea and canned goods, compared with the same period last year, more than 25% of the significant decline. In particular, fruits and nuts and grains exported 12 million yuan and 42 million yuan respectively, down by 54.8% and 43.4% respectively. Compared with last year, the main import of agricultural products increased by different degrees, especially meat, dairy and aquatic products, with imports of 11 million yuan, 92 million yuan and 21 million yuan, up 163.0%, 81.6% and 349.4%, respectively. Among imported agricultural products, fruits and grains still accounted for a large proportion, with imports of 1.74 billion and 1.96 billion, respectively, with growth rates of more than 5%.

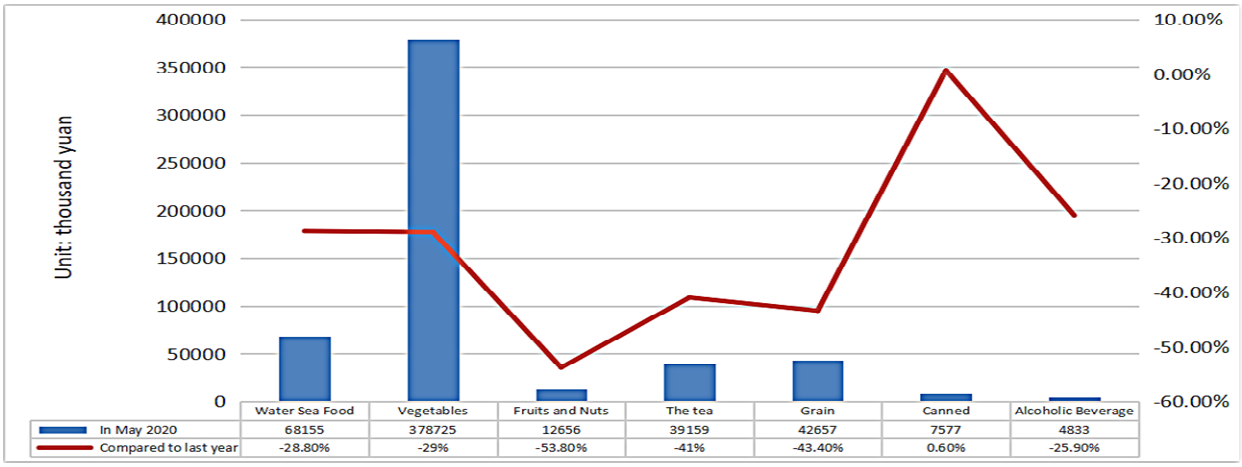

Figure 5. Main agricultural products exported by Guangxi to Asean in May, 2020

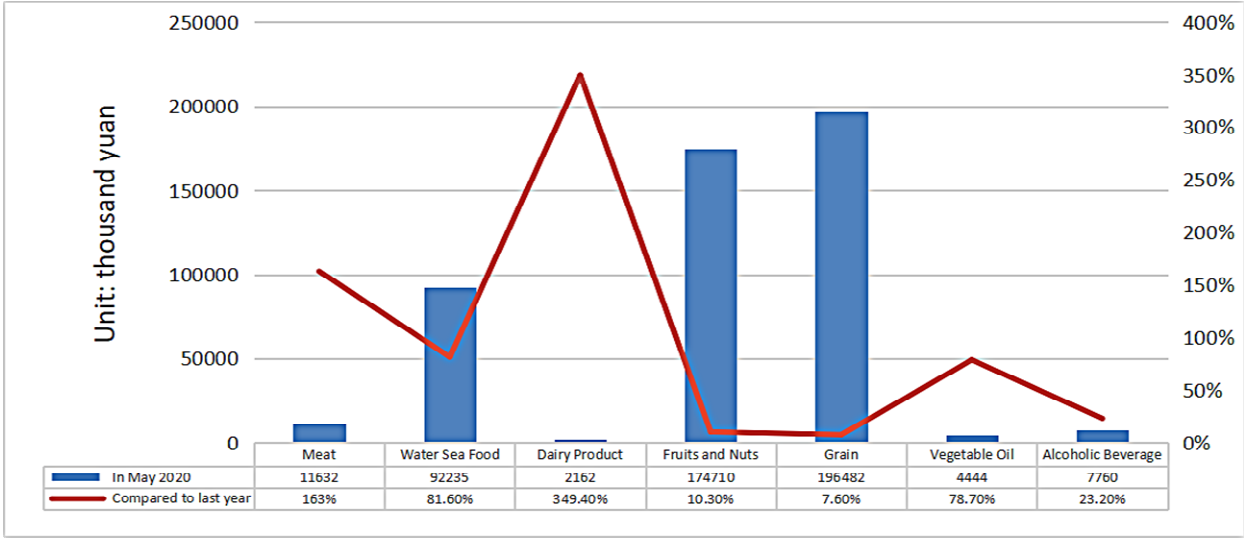

Figure 6. Main agricultural products imported from Guangxi to Asean in May, 2020

Data source: Nanning Customs [6]

3. GUANGXI'S EXPOSURE TO THE TRADE OF ASEAN AGRICULTURAL PRODUCTS IN THE PANDEMIC

3.1. The pandemic has exacerbated the trade deficit

Before being affected by the pandemic, as The scale of Guangxi's agricultural trade with Asean continues to expand, the problem of trade deficit has already existed. The issue of trade deficits is even more pronounced due to the impact of Covid - 19. In Figure 2, we can clearly see that both imports and exports, all present a certain growth trend, but imports since February 2021 and show great growth trend, in May 2021 to Asean countries in Guangxi agricultural imports more than $300 million, and guangxi exports to Asean countries although there are certain growth trend, but compared with the amount of imports is minimal. As can be seen from Figure 4, Guangxi's import of major agricultural products has increased significantly, among which the increment of dairy products even reached 348% in the same period last year. On the one hand, the geographical location and climate conditions of Guangxi and Asean countries are similar, which leads to a high degree of overlap of crops produced in Guangxi. Moreover, Asean countries have more advantages in agricultural products export due to cheaper labor and superior geographical location. On the other hand, Guangxi is highly dependent on the Vietnamese market, and Vietnam's foreign trade is highly dependent on Guangxi ports [7]. However, due to the pandemic, Vietnam's border trade is greatly restricted. Although the pandemic situation is gradually stabilizing and the customs clearance of goods at border ports is gradually recovering, the recovery of small trade and mutual trade is slow due to the strengthening of pandemic prevention and control and the lack of recovery of border logistics markets. As a result, the import market of agricultural products in Guangxi has become vacant. However, the demand for agricultural products is also increasing after the pandemic. Combined with the above reasons, the export of agricultural products in Guangxi has been greatly hit, and at the same time, the import of foreign agricultural products has increased, which may lead to the worsening of trade deficit.

3.2. Agricultural products have low added value and are more likely to be replaced

Most of Guangxi's agricultural products exported to The Asean region have low added value, which increases the possibility of loss of international orders due to the pandemic, thus

increasing the possibility of substitution of agricultural products exported [6]. Because of Guangxi agricultural exports to Asean countries in most of them are just after simple processing of native products, leading to low value-added products accounted for export agricultural products is higher, most of the primary agricultural products, and compared with the developed areas, most of guangxi agricultural products depend on the small and medium-sized agricultural products processing enterprises, leading to lack of deep processing of agricultural products, rough machining mode. Most of the processing of agricultural products is low, leading to the enterprise get more meager profits [6]. At the same time, in Asean countries and regions, due to the lack of brand, lack of quality assurance and reputation and other problems, Guangxi agricultural products acceptance and recognition is not high. In addition, the impact of the pandemic is also emerging. Asean countries have announced to strengthen quarantine and trade control on agricultural products, which makes it more difficult to import and export agricultural products, and makes it more likely for Guangxi foreign trade enterprises to lose international orders.

3.3. Agricultural products and related industries lag behind

The development of Guangxi agricultural products industry is relatively slow. As a large agricultural province in China, Guangxi's agricultural products are mainly produced by planting industry, which accounts for half of guangxi's agricultural products. Although crop yields have been rising in recent years, the increase has been slow and yields of key cash crops such as sugar cane have fluctuated. This is mainly due to the impact of imported sugar, leading to the domestic sugar market price fluctuations. At the same time, the production mode of agricultural products in Guangxi is mainly based on scattered planting of small farmers and households, which is difficult to form a scale, which leads to the existence of low production efficiency and low risk resistance ability. In addition, the industrial development of Guangxi is relatively backward, the processing rate of agricultural products is low, and the logistics and transportation industry is not developed, leading to the quality of agricultural products in the process of agricultural transportation can not be guaranteed. In terms of the way of trade, Guangxi's foreign trade mainly relies on small border trade, and its foreign trade market is relatively single, with Vietnam as the top market, accounting for up to three-quarters of the total.

4. COUNTERMEASURES AND SUGGESTIONS

4.1. We will strengthen trade consultations and stabilize the import and export of agricultural products

Chinese Premier Li Keqiang on April 14, 2020 [6]. During the extraordinary Leaders' Meeting of Asean, China, Japan and the ROK on Covid 19, China, Japan and the ROK proposed to further promote regional economic integration, further reduce tariffs, remove barriers, promote unimpeded trade, promote investment and open markets to each other. In short, it is to maintain the necessary flow of people and logistics, stable supply chain. This is a good reference and guidance for agricultural trade between Guangxi and Asean. On the basis of the China-Asean Free Trade Area, China will take fighting the pandemic as an important task in jointly building the Belt and Road Initiative with Asean countries, and comprehensively strengthen exchanges and cooperation with other countries. Relevant exchanges and cooperation should not only be limited to the fields of medical care and public health security, but also should focus on exchanges and cooperation between agriculture to ensure the stability of regional agricultural supply. For example, measures such as border trade closure and trade restrictions during pandemic

prevention and control should not be dealt with at the same time, but the prevention and control mechanism of joint countries should be improved, so as to ensure both pandemic prevention and control and regular customs clearance of agricultural products.

4.2. We will gradually establish a joint prevention and control mechanism to enhance our ability to withstand risks

Until now, the Covid -19 pandemic is still raging. On April 13, 2021, Thailand officially declared the third wave of the pandemic as nearly 1,000 confirmed cases were confirmed for three consecutive days, which can be said to be a grim situation. Therefore, taking pandemic prevention and control as the primary task is the first element of trade between China and Asean countries [6]. However, we need to think about how to ensure trade between countries while strengthening pandemic prevention and control efforts. In the author's opinion, it can be carried out by establishing regional joint protection and prevention mechanism. Through the timely transmission of information and policies, improve the ability to resist risks. Despite Asean's call for concerted action to address the pandemic collectively, there has been little coordination among member states. In addition, as Asean countries continue to strengthen pandemic prevention and control measures, there will be a series of problems in the import and export between Asean countries, for example, the export will continue to decline for a period of time, and the backlog of goods; import will face port shutdown, shipping delay, supply is not timely and other problems; therefore, it is very important to establish an effective and timely regional joint protection and prevention mechanism. Through the establishment of regional joint control and prevention mechanism, the timeliness of information circulation among countries and the openness and transparency of agricultural investment and agricultural trade policies between countries are conducive to the adjustment of import and export plans and foreign investment and trade.

4.3. With the help of e-commerce platform, improve the structure of industrial chain and enhance brand awareness

Amid the dramatic impact of Covid - 19, China is the only country to maintain positive economic growth in 2020. Among them, the rapid development of China's e-commerce trade, offer great help to China's economic development, and cross-border e-commerce, as its extension also thrive in recent years, of which agricultural products in cross-border e-commerce dealer market performance significantly, although during the pandemic prevention and control cross-border business is limited by certain resistance and e-commerce, the development of the overall trend is very obvious. Under the impact of the pandemic, the domestic and foreign sales of agricultural products in Guangxi have been hit by a huge blow, coupled with the stagnation of business contacts and the shortage of artificial harvesting, resulting in a large number of mature agricultural products, such as mandarin oranges and sugar oranges, falling prices and unsalable phenomenon. Though, the timely issued policy incentives to help agricultural extension online sales channels, the author thinks that the Guangxi agricultural products appear such circumstance, on the one hand, in addition to the disease caused by the traffic inconvenience, on the other hand is due to the agricultural products in guangxi are lack of brand and marketing, sales channels of a single combined with agricultural products processing system is not mature enough and the imperfection of the related industrial chain structure, resulting in lack of the ability to resist risks. Agricultural products in Guangxi should, therefore, with the help of a second outbreak of the opportunity of national policy support, integrated into the

"Guangdong, HK &Macau" big bay industrial chain, with the help of "Guangdong, HK &Macau" big bay area of mature system perfecting its agricultural product industry chain structure, make the agricultural products processing system to get fast development, and improve the quality of agricultural products in guangxi, and then with the help of the Internet, electric business platform for propaganda, expand sales channels, improve brand awareness and enhance agricultural products import and export trade initiatives. In this way, can make Guangxi agricultural products get benign development.

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